NUCLEOPHILIC SUBSTITUTION REACTIONS OF THIAMIN, 1'-METHYLTHIAMINIUM ION AND DERIVATIVES IN METHANOL: KINETICS AND MECHANISM

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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To My Wife, Teresa

and My Daughter, Molly

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Major Department: Chemistry

Substitution reactions of thiamin and 1'-methylthiaminium ion analogs in which a side-chain leaving group is replaced by a nucleophile were studied in methanol. Kinetic measurements using phenol analogs in methanolic piperidine and 4-amino pyridine buffers were made at 25.0 and 40.0 °C. Reactions followed second-order kinetics, first-order in substrate and in methoxide ion concentrations. Products contained the buffer base at the side-chain position. These observations require separate rate and product determining steps with one or more intermediates. A mechanism is proposed in which methoxide ion attacks C-6 of the pyrimidinium ring followed by leaving group expulsion to generate an intermediate which is captured by nucleophiles. A moderate leaving group effect was observed; rates are retarded with increasing pKa of the departing phenol. No evidence of general base catalysis was found.

To determine methoxide ion concentrations, pKa values for the buffer bases, piperidine and 4-aminopyridine, were determined at 25.0 and 40.0 °C. The method of determination employed a conventional combination electrode and pH meter to determine pH. Using pH values and buffers of known composition, the pKa's were determined.

Synthetic aspects of the substitution of 1'-methylthiaminium ion were explored focusing on the range of nucleophiles which undergo substitution. Products were synthesized which contained phenol, thiophenol, pyridine, triphenylphosphonium and many other groups. Cyclization reactions occur with 1,3-ambident nucleophiles, such as 2-aminopyridine, to produce highly fluorescent tricyclic compounds. These compounds have three fused rings in an anthracene configuration. Structures were assigned based on proton nuclear Overhauser effects, x-ray analysis and independent synthesis.

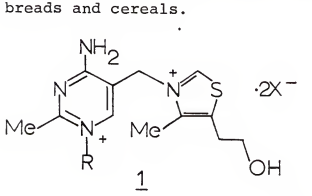
A further class of compounds was synthesized wherein the amino group of 1'-methylthiaminium ion and analogs is converted to a carbonyl function. The compounds are 1'-methyl analogs of oxythiamin, a known B_1 antagonist.

CHAPTER 1

INTRODUCTION

Thiamin (1, PmTh), or vitamin B_1, has been the subject of intense research efforts since the turn of the century.[1-6] Isolated from rice bran in 1911,[7] thiamin was shown to be active in the prevention and cure of beriberi. Research efforts over the next thirty years established dietary thiamin deficiency as the cause of the disease. Although seldom encountered today, beriberi was responsible for the deaths of many thousands of Japanese each year between 1900 and 1940.[2] It is little wonder that the Japanese dominate the literature concerning thiamin. Since the time of its isolation, thiamin has been characterized,[8,9,10] synthesized,[11,12,13] and its chemistry extensively studied *in vivo* and *in vitro*.[1-6]

The structure of thiamin consists of a 4-amino-2-methylpyrimidine ring bridged at the 5 position by a methylene group to 5-(2-hydroxyethyl)-4-methylthiazole. It is often encountered as the chloride hydrochloride (1a, X = Cl^-) or as the mononitrate free base (1a, X = NO_3^-) in fortified breads and cereals.

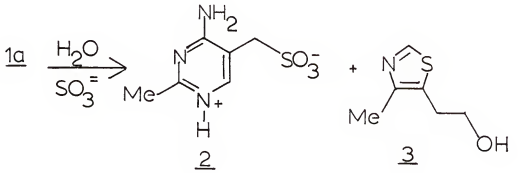


1

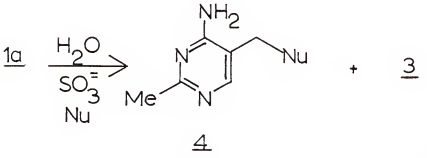
1a R = H (PmTh)

1b R = Me (NMePmTh)

In 1935 Williams and co-workers established the structure of thiamin by identifying the products of reaction with sulfite ion.[8-10] Thiamin is cleaved by sulfite ion to give the 5-methylsulfonic acid derivative (2, $PmSO_3^-$) and the corresponding thiazole (3, Th), Equation 1. Sulfite ion also was found to catalyze a related reaction in which nucleophiles substitute at the methylene position forming 4 (PmNu) and releasing 3, Equation 2. In the absence of sulfite ion, no substitution occurs over a similar time span.[14-18]



Equation 1



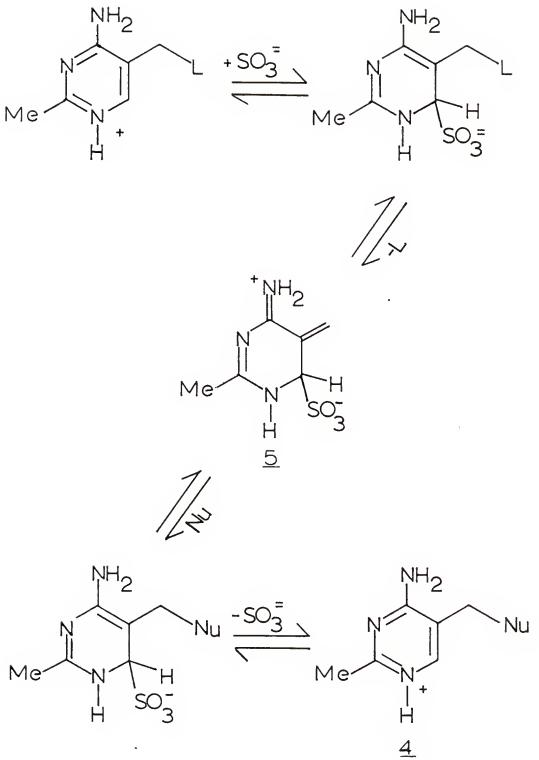
Equation 2

The mechanism of nucleophilic substitution catalyzed by sulfite ion was the subject of many investigations,[19] without satisfactory resolution. In 1976 Zoltewicz and Kauffman postulated a novel mechanism consistent with their kinetic

observations and those of previous workers.[20] The mechanism involves initial sulfite ion attack at the 6 position of the protonated pyrimidine ring followed by expulsion of the leaving group L. Nucleophiles then compete for intermediate 5 to form the products, 4 and L, as shown in Scheme 1. Subsequent investigations with thiamin, the 1'-methylthiaminium ion (1b) and derivatives have substantiated this mechanism.[21-25]

Thiaminase I is an enzyme which destroys thiamin.[26-29] It is found in freshwater fish, shellfish (especially clams) and ferns. Ingestion of raw clams, certain fish and ferns can produce beriberi-like conditions in man and animals. Studies performed *in vitro* revealed that the enzyme replaces the thiazole portion of thiamin with nucleophiles in a reaction reminiscent of nucleophilic substitution by sulfite ion. Zoltewicz and Kauffman suggested the sulfite ion cleavage mechanism as a model for thiaminase I action.[20]

Shimahara and co-workers observed a direct nucleophilic substitution reaction between thiamin and pyridine (6, Py) in 95% methanol without the use of sulfite ion as a catalyst but failed to note the significance of this unusual phenomenon.[30] We therefore investigated the mechanism of nucleophilic substitution reactions of thiamin, the 1'-methylthiaminium ion (1b, NMePmTh) and their derivatives by nucleophiles in methanol. The present investigation of the kinetics of substitution is divided into three parts and is reported in Chapter 3. The first section is a study of the cleavage of a series of substituted phenol derivatives of the 1'-methylthiaminium ion in methanolic piperidine and



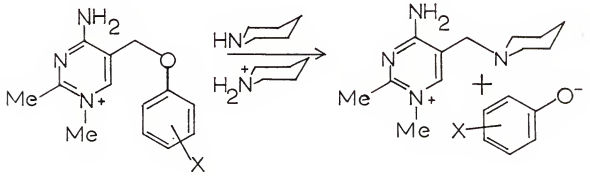
Scheme 1

4-aminopyridine buffers at 40.0 °C. The substituents include para-nitro, para-cyano, meta-chloro and the parent phenol; the derivatives are abbreviated NMePmOØpNO$_2$ (7), NMePmOØpCN (8), NMePmOØmCl (9), and NMePmOØH (10), respectively. In all cases the rate of reaction is dependent on the concentrations of substrate and of methoxide ion as expressed in Equation 3.

$$\text{rate} = k_2\,[CH_3O^-]\,[\text{NMePmOØx}] \qquad \text{Equation 3}$$

The determination of the methoxide ion concentration in the piperidine and 4-aminopyridine buffers used in kinetic runs necessitates the determination of pKa values and is the subject of Chapter 2.

No general base or nucleophilic catalysis was discovered. The rate dependence on leaving group was found to be para-nitro > para-cyano > meta-chloro > hydrogen for the series of phenols. Although the reaction rate is dependent on methoxide ion concentration, the product of substitution has the buffer base bonded to the methylene group, Equation 4, thus requiring the presence of an intermediate during the substitution process.



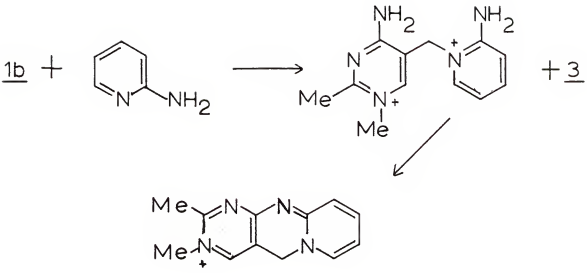
Equation 4

The mechanism probably is analogous to the aqueous sulfite ion mechanism with methoxide ion acting as catalyst in place of sulfite ion.

The second part of the study utilizes unbuffered solutions of 4-aminopyridine and sodium azide to effect substitution on $NMePmO\emptyset pNO_2$ (7) at 25 °C. The results of the study indicate that the rate of reaction is dependent on the nucleophile concentration in a half-order fashion as expected from the results found with buffered solutions.

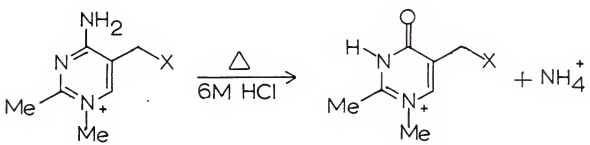
The third section describes the kinetics of substitution of thiamin (1a, PmTh) and 1-[(4-amino-2-methyl-5-pyrimidinyl)methyl]pyridinium chloride (11, PmPy) by nucleophiles at 71.5 °C in methanol. Results indicate that the reaction is first-order in substrate alone. The postulated mechanism requires both methoxide ion and proton for these substrates. The requirement of proton and methoxide ion is kinetically indistinguishable from that of solvent methanol alone.

Chapter 4 deals with syntheses and explores the range of nucleophiles which substitute 1'-methylthiaminium ion. Nucleophiles include phenols, thiophenols, pyridines, triphenylphosphine and azide ion. Ambident nucleophiles such as 2-aminopyridines, 2-aminothiazole, and thiourea represent a special class of reactions as cyclization follows substitution forming highly fluorescent bicyclic and tricyclic compounds as shown in Equation 5. The structures of the tricyclic compounds were identified by proton-proton nuclear Overhauser experiments, x-ray analysis, ^{15}N and ^{13}C magnetic resonance.



Equation 5

The hydrolysis of 1'-methylthiaminium ion derivatives to 1'-methyloxythiaminium ion derivatives is also discussed in Chapter 4. Heating 1'-methylthiaminium ion derivatives in 6M HCl results in the replacement of the 4-amino group of the pyrimidinium ring to give a carbonyl function, Equation 6. These compounds are of interest because oxythiamin is a thiamin antagonist, or anti-vitamin.[30-31] Oxy-compounds synthesized include those where X is thiazole (3), pyridine (6), 3-methylimidazole and thiophenol.



Equation 6

CHAPTER 2

pH MEASUREMENTS IN METHANOL

Kinetic studies of substitution of 1'-methylthiaminium ion derivatives in methanol, detailed in Chapter 3, show a rate dependence on the concentration of methoxide ion. In order to determine the role which methoxide ion plays in the substitution mechanism the concentration of methoxide ion in a kinetic run must first be determined.

This chapter details the problems encountered in non-aqueous pH measurement, the method of pH determination, and determination of pKa values for 4-aminopyridine and piperidine at 25.0 and 40.0 °C. Having established these pKa values, the methoxide ion concentration can be determined for kinetic runs at any buffer ratio.

Non-aqueous pH measurements are complicated by a variety of factors such as the dielectric constant of the solvent, solvent-solute interactions, and ionic strength effects. The importance of these factors depends on the particular solvent.[32] According to one approach measurements are made in mixed aqueous-organic solvents or pure organic solvents by initial calibration of the pH meter with aqueous buffers and application of the calibrated electrode to the solvent of interest.[33] While such measurements may be useful for practical work, they lack thermodynamic significance. Research efforts by two groups, Bates and co-workers[34] and

de Ligny and co-workers,[35-42] established a pH scale for some non-aqueous media consistent with thermodynamic equations for acid-base equilibria.

Ritchie and co-workers[43-45] have pioneered practical pH measurement in methanol and have determined pKa values for primary pH standards, some of which are given in Table 1. The pKa values in Table 1 were determined at 1.00×10^{-3} M ionic strength and are uncorrected for ion activity effects which are small. The method used by Ritchie and co-workers employs a calomel electrode filled with methanolic potassium chloride,[46] a glass electrode and a conventional pH meter. We adopted a similar approach and used 4-picoline and 1,4-diazabicyclo[2.2.2]octane (Dabco) as primary pH standards with pKa values at 25.0 °C of 6.09 and 8.99, respectively, as given by Ritchie and co-workers.

As our kinetic studies were conducted both at 25.0 and 40.0 °C, it was necessary to establish primary pH standards at both temperatures. However, Ritchie and Heffley[43] noted that little data exist for the temperature dependence of pKa values in methanol. They established free energy, enthalpy and entropy terms, Table 2, for the ionization of 4-picoline in methanol, thereby permitting the calculation of the pKa of 4-picoline at 40.0 °C from standard thermodynamic equations, 7 and 8.

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \qquad \text{Equation 7}$$

$$pKa = \frac{\Delta G^\circ}{2.303\ RT} \qquad \text{Equation 8}$$

Table 1. pKa Values for Calibrating Buffers in Methanol at 25.0 °C and Ionic Strength of 1.00×10^{-3} M.

Compound	pKa
4-Picoline[a]	6.09
Salicylic Acid[b]	7.53
Dabco[c]	8.99
Triethylamine[c]	10.88
Ethylenediamine[d]	10.90

[a] Ref. 43.

[b] Ionic strength at 1.00×10^{-2} molal, Ref. 41.

[c] Ref. 45.

[d] Ref. 44.

Table 2. Temperature Dependence of the pKa of 4-Picoline in Methanol.[a]

pKa, 25.0 °C	pKa, 40.0 °C	ΔpKa	ΔH°, Kcal/mole	-ΔS°, cal/deg mole
6.09	5.82	0.27	6.7	5.2

[a] Ref. 43.

Table 3. Temperature Dependence of the pKa's of Amines in Water.

Amine	pKa, 25.0 °C	pKa[a], 40.0 °C	ΔpKa	ΔH°, Kcal/mole	-ΔS°, cal/deg mole
4-Picoline[b]	6.00	5.74	0.26	7.56	2.1
Dabco[c]	8.82	8.58	0.24	7.30	15.9
4-Aminopyridine[d]	9.11	8.72	0.39	11.25	3.94
Triethylamine[c]	10.75	10.40	0.35	10.38	14.4
Piperidine[e]	11.12	10.67	0.45	———	———

[a]Calculated from thermodynamic values data.

[b]Ref. 45,47.

[c]Ref. 48.

[d]Ref. 49.

[e]Ref. 50.

Table 4. pKa Differences Between Methanolic and Aqueous Amine Solutions at 25 °C.

Amine	ΔpKa[a]
4-Picoline	0.09
Dabco	0.17
Triethylamine	0.13
Ethylenediamine[b]	1.03

[a]Calculated from Tables 1 and 3.

[b]Aqueous pKa 9.87.

They also stated that there is little difference between pKa values for tertiary amines in aqueous and in methanolic solution. Table 3 shows this difference for the primary pH standards of Table 1. Furthermore, the change in pKa with temperature for 4-picoline in methanol is almost the same as that in water, 0.27 and 0.26, respectively, Tables 2 and 4. Therefore, it appears reasonable to apply the pKa temperature correction for a given tertiary amine in water from Table 4 to the pKa for that amine in methanol, Equation 9.

$$pKa_{40\ °C,\ MeOH} = pKa_{25\ °C,\ MeOH} - \Delta pKa_{aq(25\text{-}40\ °C)}$$

Equation 9

Application of Equation 9 to the primary pH standards for this work, Dabco and 4-picoline, gives their pKa values as 8.75 and 5.82, respectively, in methanol at 40.0 °C.

The pH values of a series of piperidine and 4-aminopyridine buffers were measured at 25.0 and 40.0 °C using 4-picoline and Dabco buffers as primary pH references. Measurements were made at ionic strengths of 1.00×10^{-2} and 1.00×10^{-3} M as determined by the concentration of the buffer conjugate acid and at 9.48×10^{-3} M maintained with both benzyltriethylammonium perchlorate (BTAP) and buffer. The high and low pH readings from several measurements were converted to hydrogen ion concentrations, and Ka values were calculated using this value and the buffer ratio according to Equation 10.

$$Ka = \frac{[H^+][B]}{[BH^+]}$$ Equation 10

All Ka values for a buffer series then were averaged to give a mean Ka value from which the pKa was calculated. Error limits are given as the standard deviation of the Ka value. The buffer stoichiometry, pH readings, hydrogen ion concentrations, Ka values and pKa's are recorded in Tables 5 through 18. Solvolysis corrections of the concentrations of buffer acid and base are insignificant.

The pKa values for piperidine and 4-aminopyridine at 25.0 and 40.0 °C and ionic strengths of 1.00×10^{-2} and 1.00×10^{-3} M $[BH^+]$ and 9.48×10^{-3} M [BTAP] are summarized in Table 19. These values were corrected for ionic strength dependence with the Debye-Hückel treatment, Equation 11,[51] where γ is the activity coefficient, Zi is the charge of an ion, I is the ionic strength in molar units of the univalent buffer acid, D is the dielectric constant of methanol,[52] T is the absolute temperature in °K, and å is the ion size parameter in Angstroms.

$$-\log \gamma := \frac{(1.82455 \times 10^6)\ Zi^2\sqrt{I}}{(DT)^{3/2}\ [1 + 50.2904(DT)^{-1/2}\ \text{å}\sqrt{I}]}$$

Equation 11

Ion size parameters for the piperidinium and 4-aminopyridinium cations were estimated to be 4.5 and 5.0 Å by comparison with the diethylammonium cation (4.5 Å) and the para-chlorobenzoate anion (5.0 Å); the latter values were determined by Kielland.[53]

The measured pKa values, Debye-Hückel corrections and corrected pKa values are presented in Table 19. Even after making the activity corrections, pKa values for 10^{-2} and 10^{-3} M ionic strengths differed slightly, about 0.1-0.2. This observation will be discussed later. The pKa values determined at 10^{-2} and 10^{-3} M ionic strength were averaged to produce operational pKa values as shown in Table 20. These operational pKa values were employed to calculate pK and methoxide ion concentrations for the buffered kinetic runs of Chapter 3 using the stoichiometric buffer ratios employed for the kinetic runs according to Equations 12, 13, and 14.

$$pH = pKa + \log \frac{[B]}{[BH^+]} \qquad \text{Equation 12}$$

$$pOCH_3 = pKs - pH \qquad \text{Equation 13}$$

$$[CH_3O^-] = \text{antilog } (-pOCH_3) \qquad \text{Equation 14}$$

The pKs of methanol is 16.92 and 16.56 at 25.0 and 40.0 °C respectively.[54,55] The use of the operational pKa value is apparently justified by the fact that the kinetic studies of Chapter 3, using 10^{-2} and 10^{-3} M ionic strength buffers, give observed rate constants which are the same within experimental error. The implication is that the methoxide ion concentration is essentially the same for these solutions.

The pKa values of tertiary amines at temperatures other than 25 °C were calculated by applying the temperature correction for aqueous solution to methanol. This difference in pKa at 25 and 30 °C for piperidine and 4-aminopyridine in water is 0.45 and 0.39, respectively, Table 3. The

observed change in pKa with temperature measured by our methods using methanol is 0.31 and 0.25 for piperidine and 4-aminopyridine. The difference between the calculated change based on water and that found experimentally for methanol is 0.15 for both amines. This lack of agreement could be due in part to an error in the estimate of the pKa at 40 °C for the Dabco calibrating buffer, the pKa value of 4-picoline in MeOH at 40 °C being known. If the value for Dabco were in error then one would expect the ΔpKa value to be larger for piperidine than for 4-aminopyridine, because 4-aminopyridine has a pKa value closer to that of the calibrating Dabco buffer than does piperidine. Justification for the validity of the pKa values employed in pH calculations in kinetic runs lies in the constancy of second order rate constants obtained for the same substrate using both piperidine and 4-aminopyridine buffers.

For a given pKa determination, Tables 5 through 18, there is a systematic trend; the Ka values calculated from Equation 10 increase as the concentration of amine base increases. Buffers prepared by Method I range in composition from 5 to 50% ionization or buffer ratios of 20/1 to 1/1 defined by $[B]/[BH^+]$. Using the Ka value for a 1/1 buffer ratio as a reference, the deviation of the Ka value at a 20/1 ratio can be calculated. A survey of these values shows that such a deviation can be as small as -3 to 3%, essentially experimental error (Tables 13 and 16; 4-aminopyridine, 10^{-2} M ionic strength, 25 and 40 °C) or as large

as 40% (Tables 7 and 14: piperidine, 10^{-3} M ionic strength, 25 °C; 4-aminopyridine, 10^{-2} M ionic strength, 25 °C). Temperature, ionic strength or the identity of the amine does not appear to influence the deviation consistently. Buffers prepared by Method II range from 20 to 80% ionization or buffer ratios of 5/1 to 1/5 as defined above. The calculation of the extent of deviation for these buffers compares Ka values obtained at a buffer ratio of 5/1 to those obtained at buffer ratio of 1/5 taken as a standard. These buffers show deviations of 12 and 36% (Tables 8 and 12: piperidine, 10^{-3} M ionic strength, 25 and 40 °C). The deviation in Ka values for all determinations ranges from 0 to 40% with an average of 19%. This results in an uncertainty in pKa values of ±0.02 to ±0.06 with an average deviation of ±0.04.

There is a curious effect exhibited in the pKa values of Table 19 associated with the manner in which the ionic strength is maintained. The pKa values determined for piperidine at 25.0 °C serve as an example. When the ionic strength is maintained at 10^{-3} M by the conjugate acid of the buffer the pKa corrected for ion activity is 10.94. Increasing the ionic strength to 10^{-2} M with benzyltriethylammonium perchlorate gives, after activity correction, a pKa of 10.95, identical within experimental error. The anomaly occurs when the ionic strength is maintained at 10^{-2} M with the conjugate acid of the buffer. The pKa, after activity correction, is determined to be 11.10, 0.15 pK

high. The factor of 0.15 pK is also seen at 40.0 °C. The same anomaly occurs to a lesser extent with 4-aminopyridine; the pKa values determined at 10^{-2} M ionic strength based on the conjugate buffer acid are 0.07 high.

At high pH the glass electrode becomes inaccurate as sodium ions penetrate the glass membrane. This effect is commonly known as sodium ion error.[51,56,57] Little is known however about the magnitude of such a sodium ion error in methanol. We observed that the Radiometer GK2321C combination electrode is subject to sodium ion error in methanol above pH 11. The use of sodium perchlorate to maintain the ionic strength of 10^{-2} M in preliminary determinations of the pKa of piperidine caused deviations of 50 to 60% in the Ka values as determined by our method. The nature of the deviation is consistent with a sodium ion error in that as the concentration of piperidine is increased, the measured pH is lower than predicted, for example, pKa values calculated from buffer ratios and measured pH deviate by 0.18 pK as the buffer ratio is changed from 20/1 to 1/1, $[B]/[BH^+]$. Verification of the sodium ion error comes from the use of a Radiometer GK2401B combination electrode which is not as sensitive to sodium ions. The Ka and pKa values obtained with this electrode do not show the large deviations found for the 2321C electrode. Also, the 2321C electrode produced stable Ka values when the large organic cation benzyltriethylammonium perchlorate was used in place of sodium perchlorate.

Glass electrode membranes are dehydrated by non-aqueous media. Dehydration can result in unstable or erratic pH measurements.[51] The membrane of the 2401B electrode tends to dehydrate much more rapidly than the 2321C membrane, one day as opposed to two weeks. For this reason, the 2321C electrode was used for routine pH measurements. Maintaining ionic strength with electrolytes other than the buffer conjugate acid was accomplished with BTAP thereby avoiding sodium ion error.

As a check on the pH calibration, the pH of a 1/1 salicylic acid buffer (0.01 molal) was employed and was found to be in agreement, giving a pH reading of 7.50. This is to be compared to the literature value, 7.53.[41] Other buffers proved to be unsatisfactory. Thus, a 1/1 ethylenediamine buffer, ionic strength at 10^{-3} M, gave a pH reading of 10.79 (10.90 lit.[45]), low by 0.11. This primary diamine is very hygroscopic and, although distilled twice from sodium, could have picked up water. Moreover, primary amines are known to show a pronounced difference in pKa values between water and methanol, Table 3. The pKa value of triethylamine could not be reproduced either. A 1/1 buffer at 10^{-3} M ionic strength gave a pH reading of 10.45 (10.88 lit.[44]). This deviation is probably due to water; the triethylamine used was not distilled before use and the stoichiometric concentration of the base was probably in error. Ritchie has proposed the use of potassium hydrogen phthalate (KHP)[44] at a concentration of 0.0146 M

in methanol as a primary pH reference. His value of 8.99 (identical to the Dabco value) could not be reproduced. Our value determined for KHP was 8.80. No explanation can be given.

In summary Ritchie's method of pH measurement in methanol was used to determine the hydrogen ion concentration of a series of piperidine and 4-aminopyridine buffers at 10^{-2} and 10^{-3} M ionic strengths and at 25.0 and 40.0 °C. These pH values were used to determine the Ka and pKa values. Pronounced ionic strength effects were noted as well as sodium ion error.

Table 5. Determination of the pKa of Piperidine in Methanol at 25.0 °C and 1.00±.02 x 10^{-2} M Ionic Strength[a] Using Buffer Solutions[b] and Measured pH Values.

10 [B],M	10^2 [BH^+],M	[B]/[BH^+]	pH	10^{13} [H^+],M	10^{12}Ka
2.01	1.02	19.7	12.52-12.55	3.02-2.82	5.95-5.56
1.02	1.02	10.0	12.22-12.26	6.03-5.50	6.03-5.50
0.520	0.999	5.21	11.95-11.98	12.2-10.5	5.85-5.46
0.0994	1.02	0.975	11.26-11.32	55.0-47.9	5.36-4.67
				Avg 5.55±0.43 x 10^{-12}	
				pKa 11.26±0.04	

[a]Ionic strength determined by [BH^+].
[b]Buffers prepared by Method I.

Table 6. Determination of the pKa of Piperidine in Methanol at 25.0 °C and 9.48±.02 x 10^{-3} M Ionic Strength[a] Using Buffer Solutions[b], Measured pH Values, and Added Electrolyte.

10^2 [B],M	10^3 [BH^+],M	[B]/[BH^+]	pH	10^{13} [H^+],M	10^{12}Ka
2.01	1.02	19.7	12.37-12.39	4.27-4.07	8.41-8.02
1.02	1.02	10.0	12.08-12.13	8.32-7.41	8.32-7.41
0.520	0.999	5.21	11.83-11.84	14.8-14.1	7.71-7.35
0.0994	1.02	0.975	11.11-11.14	77.6-72.4	7.57-6.89
					Avg 7.71±0.52 x 10^{-12}
					pKa 11.11±0.03

[a]Ionic strength maintained with 8.48 x 10^{-3} M BTAP; [BH^+] at 1.00±.02 x 10^{-3} M.
[b]Buffers prepared by Method I.

Table 7. Determination of the pKa of Piperidine in Methanol at 25.0 °C and 1.00±.02 x 10^{-3} M Ionic Strength[a] Using Buffer Solutions[b] and Measured pH Values.

10^2 [B],M	10^3 [BH^+],M	[B]/[BH^+]	pH	10^{13} [H^+],M	10^{11}Ka
2.01	1.02	19.7	12.21-12.26	6.17-5.50	1.22-1.08
1.02	1.02	10.0	11.94-11.98	11.5-10.7	1.15-1.07
0.520	0.999	5.21	11.65-11.72	23.4-19.1	1.22-0.993
0.0994	1.02	0.975	11.05-11.08	89.1-83.2	0.869-0.811
				Avg 1.05±0.15 x 10^{-11}	
				pKa 10.98±0.06	

[a]Ionic strength determined by [BH^+].
[b]Buffers prepared by Method I.

Table 8. Determination of the pKa of Piperidine in Methanol at 25.0 °C and 1.03×10^{-3} M Ionic Strength[a] Using Buffer Solutions[b] and Measured pH Values.

10^3 [B],M	10^3[BH^+],M	[B]/[BH^+]	pH	10^{12} [H^+],M	10^{12}Ka
4.24	1.03	4.12	11.60-11.64	2.51-2.29	10.3-9.43
3.18	1.03	3.09	11.49-11.53	3.24-2.95	10.0-9.12
2.12	1.03	2.06	11.28-11.30	5.25-5.01	10.8-10.3
1.06	1.03	1.03	11.02-11.06	9.55-8.71	9.84-8.97
0.636	1.03	0.617	10.82-10.84	15.1-14.5	9.32-8.95
0.424	1.03	0.412	10.66-10.68	21.9-20.9	9.02-8.61
0.212	1.03	0.206	10.36-10.38	43.7-41.7	9.00-8.59
				Avg $9.45 \pm 0.69 \times 10^{-12}$	
				pKa 11.02±0.03	

[a]Ionic strength determined by [BH^+].
[b]Buffers prepared by Method II.

Table 9. Determination of the pKa of Piperidine in Methanol at 40.0 °C and 1.00±.02 x 10^{-2} M Ionic Strength[a] Using Buffer Solutions[b,c] and Measured pH Values.

10 [B],M	10^2 [BH^+],M	[B]/[BH^+]	pH	10^{13} [H^+],M	10^{11}Ka
2.01	1.02	19.7	12.20-12.21	6.31-6.17	1.24-1.22
1.02	1.02	10.0	11.91-11.93	12.3-11.8	1.23-1.18
0.520	0.999	5.21	11.64-11.65	22.9-22.4	1.19-1.17
0.0994	1.02	0.975	10.93-10.96	117-110	1.14-1.07
				Avg 1.18±0.06 x 10^{-11}	
				pKa 10.93±0.02	

[a]Ionic strength determined by [BH^+].
[b]Buffers prepared by Method I.
[c]Concentrations are uncorrected for the thermal expansion of methanol.

Table 10. Determination of the pKa of Piperidine in Methanol at 40.0 °C and 9.48±.02 x 10^{-3} M Ionic Strength[a] Using Buffer Solutions[b,c] Measured pH Values, and Added Electrolyte.

10^2 [B],M	10^3 [BH^+],M	[B]/[BH^+]	pH	10^{12} [H^+],M	10^{11}Ka
2.01	1.02	19.7	12.02-12.04	0.955-0.912	1.88-1.80
1.02	1.02	10.0	11.71-11.76	1.95-1.74	1.95-1.74
0.520	0.999	5.21	11.48-11.49	3.31-3.24	1.72-1.69
0.0994	1.02	0.975	10.82-10.85	15.1-14.1	1.47-1.37
				Avg 1.71±0.20 x 10^{-11}	
				pKa 10.78±0.06	

[a]Ionic strength maintained with 8.48 x 10^{-3} M BTAP; [BH^+] at 1.00±.02 x 10^{-3} M.
[b]Buffers prepared by Method I.
[c]Concentrations are uncorrected for the thermal expansion of methanol.

Table 11. Determination of the pKa of Piperidine in Methanol at 40.0 °C and 1.00±.02 x 10^{-3} M Ionic Strength[a] Using Buffer Solutions[b,c] and Measured pH Values.

10^2 [B],M	10^3 [BH^+],M	[B]/[BH^+]	pH	10^{12} [H^+],M	10^{11}Ka
2.01	1.02	19.7	11.94-11.97	1.15-1.07	2.27-2.11
1.02	1.02	10.0	11.65-11.68	2.24-2.09	2.24-2.09
0.520	0.999	5.21	11.39-11.40	4.07-3.98	2.12-2.07
0.0994	1.02	0.975	10.71-10.75	19.5-17.8	1.90-1.74
				Avg 2.07±0.17 x 10^{-11}	
				pKa 10.68±0.03	

[a]Ionic strength determined by [BH^+].
[b]Buffers prepared by Method I.
[c]Concentrations are uncorrected for thermal expansion of methanol.

Table 12. Determination of the pKa of Piperidine in Methanol at 40.0 °C and 1.03×10^{-3} M Ionic Strength[a] Using Buffer Solutions[b,c] and Measured pH Values.

10^3 [B],M	10^3[BH^+],M	[B]/[BH^+]	pH	10^{12} [H^+],M	10^{11}Ka
4.24	1.03	4.12	11.28-11.34	5.25-4.57	2.16-1.88
3.18	1.03	3.09	11.16-11.23	6.92-5.89	2.14-1.82
2.12	1.03	2.06	10.97-11.04	10.7-9.12	2.20-1.88
1.06	1.03	1.03	10.72-10.79	19.1-16.2	1.97-1.67
0.636	1.03	0.617	10.52-10.59	30.2-25.7	1.86-1.59
0.424	1.03	0.412	10.37-10.43	42.7-37.2	1.76-1.53
0.212	1.03	0.206	10.14-10.15	72.4-70.8	1.49-1.46
				Avg $1.82 \pm 0.25 \times 10^{-11}$	
				pKa 10.74±0.06	

[a] Ionic strength determined by [BH^+].
[b] Buffers prepared by Method II.
[c] Concentrations are uncorrected for the thermal expansion of methanol.

Table 13. Determination of the pKa of 4-Aminopyridine in Methanol at 25.0 °C and 1.00×10^{-2} M Ionic Strength[a] Using Buffer Solutions[b] and Measured pH Values.

10 [B],M	10^2 [BH^+],M	[B]/[BH^+]	pH	10^{12} [H^+],M	10^{11}Ka
2.00	1.01	19.8	11.30-11.32	5.01-4.79	9.92-9.48
1.01	1.00	10.1	11.00-11.01	10.0-9.77	10.1-9.87
0.501	0.999	5.02	10.70-10.71	20.0-19.5	10.0-9.79
0.103	0.999	1.03	10.03-10.05	93.3-89.1	9.61-9.18
				Avg $9.74 \pm 0.30 \times 10^{-11}$	
				pKa 10.01 ± 0.02	

[a]Ionic strength determined by [BH^+].
[b]Buffers prepared by Method I.

Table 14. Determination of the pKa of 4-Aminopyridine in Methanol at 25.0 °C and 9.48±.02 x 10^{-3} M Ionic Strength[a] Using Buffer Solutions[b], Measured pH Values and Added Electrolyte.

10^2 [B],M	10^3 [BH^+],M	[B]/[BH^+]	pH	10^{12} [H^+],M	10^{10}Ka
2.00	1.01	19.8	11.17-11.19	6.76-6.47	1.34-1.28
1.01	1.00	10.1	10.91-10.91	12.3-12.3	1.24-1.24
0.501	0.999	5.02	10.63-10.64	23.4-22.9	1.17-1.15
0.103	0.999	1.03	10.01-10.02	97.7-95.5	0.953-0.931
				Avg 1.16±0.15 x 10^{-10}	
				pKa 9.94±0.06	

[a]Ionic strength maintained at 8.48 x 10^{-3} M with BTAP; [BH^+] at 1.00±.02 x 10^{-3} M.
[b]Buffers prepared by Method I.

Table 15. Determination of the pKa of 4-Aminopyridine in Methanol at 25.0 °C and 1.00×10^{-3} M Ionic Strength[a] Using Buffer Solutions[b] and Measured pH Values.

10^2 [B],M	10^3 [BH^+],M	[B]/[BH^+]	pH	10^{12} [H^+],M	10^{10}Ka
2.00	1.01	19.8	11.09-11.10	8.13-8.94	1.61-1.57
1.01	1.00	10.1	10.81-10.84	15.5-14.5	1.57-1.46
0.501	0.999	5.02	10.55-10.56	28.2-27.5	1.42-1.38
0.103	0.999	1.03	9.91-9.93	123-117	1.25-1.14
				Avg $1.43 \pm 0.17 \times 10^{-10}$	
				pKa 9.84±0.04	

[a]Ionic strength determined by [BH^+].
[b]Buffers prepared by Method I.

Table 16. Determination of the pKa of 4-Aminopyridine in Methanol at 40.0 °C and 1.00±.01 x 10^{-2} M Ionic Strength[a] Using Buffer Solutions[b,c] and Measured pH Values.

$10\ [B],M$	$10^2\ [BH^+],M$	$[B]/[BH^+]$	pH	$10^{12}\ [H^+],M$	$10^{10}Ka$
2.00	1.01	19.8	11.05-11.06	8.91-8.71	1.76-1.72
1.01	1.00	10.1	10.74-10.78	18.2-16.6	1.84-1.68
0.501	0.999	5.02	10.42-10.45	38.0-35.5	1.91-1.78
0.103	0.999	1.03	9.76-9.76	174-174	1.79-1.79
				Avg 1.78±0.07 x 10^{-10}	
				pKa 9.75±0.04	

[a] Ionic strength determined by $[BH^+]$.
[b] Buffers prepared by Method I.
[c] Concentrations are uncorrected for the thermal expansion of methanol.

Table 17. Determination of the pKa of 4-Aminopyridine in Methanol at 40.0 °C and 9.48±.02 x 10^{-3} M Ionic Strength[a] Using Buffer Solutions[b,c] Measured pH Values and Added Electrolyte.

10^2 [B],M	10^3 [BH^+],M	[B]/[BH^+]	pH	10^{11} [H^+],M	10^{10}Ka
2.00	1.01	19.8	10.94-10.96	1.15-1.10	2.28-2.18
1.01	1.00	10.1	10.67-10.69	2.14-2.04	2.16-2.06
0.501	0.999	5.02	10.38-10.39	4.17-4.07	2.09-2.04
0.103	0.999	1.03	9.73-9.75	18.6-17.8	1.92-1.83
				Avg 2.07±0.14 x 10^{-10}	
				pKa 9.68±0.03	

[a]Ionic strength maintained at 8.48 x 10^{-3} M with BTAP; [BH^+] at 1.00±.02 x 10^{-3} M.
[b]Buffers prepared by Method I.
[c]Concentrations are uncorrected for the thermal expansion of methanol.

Table 18. Determination of the pKa of 4-Aminopyridine in Methanol at 40.0 °C and 1.00×10^{-3} M Ionic Strength[a] Using Buffer Solutions[b,c] and Measured pH Values.

10^2 [B],M	10^2 [BH^+],M	[B]/[BH^+]	pH	10^{11} [H^+],M	10^{10}Ka
2.00	1.01	19.8	10.85-10.85	1.41-1.41	2.79-2.79
1.01	1.00	10.1	10.57-10.58	2.69-2.63	2.72-2.66
0.501	0.999	5.02	10.31-10.32	4.90-4.79	2.46-2.40
0.103	0.999	1.03	9.63-9.66	23.4-21.9	2.41-2.26
				Avg $2.56 \pm 0.20 \times 10^{-10}$	
				pKa 9.59 ± 0.03	

[a]Ionic strength maintained at 8.48×10^{-3} M with BTAP; [BH^+] at $1.00 \pm .02 \times 10^{-3}$ M.
[b]Buffers prepared by Method I.
[c]Concentrations are uncorrected for the thermal expansion of methanol.

Table 19. Debye-Hückel Corrected pKa Values for Piperidine and 4-Aminopyridine in Methanol.

Base	Temp. °C	Ionic Strength, M[a]	Debye-Hückel[b] Correction	pKa Measured	pKa Corrected
piperidine	25.0	1.00×10^{-2}	0.155	11.26	11.10
		[c] 9.48×10^{-3}	0.155	11.11	10.95
		1.00×10^{-3}	0.056	11.00	10.94
	40.0	1.00×10^{-2}	0.144	10.93	10.79
		[c] 9.48×10^{-3}	0.144	10.78	10.64
		1.00×10^{-3}	0.052	10.71	10.66
4-aminopyridine	25.0	1.00×10^{-2}	0.152	10.01	9.86
		[c] 9.48×10^{-3}	0.152	9.94	9.79
		1.00×10^{-3}	0.056	9.84	9.78
	40.0	1.00×10^{-2}	0.142	9.75	9.61
		[c] 9.48×10^{-3}	0.142	9.68	9.54
		1.00×10^{-3}	0.052	9.59	9.54

[a] Ionic strength determined by $[BH^+]$.
[b] Calculated from Equation 11.
[c] Ionic strength maintained with 9.48×10^{-3} M BTAP; $[BH^+]$ at 1.00×10^{-3} M.

Table 20. Operational pKa Values for Piperidine and 4-Aminopyridine in Methanol.

Base	Temp. °C	pKa
piperidine	25.0	11.02
	40.0	10.73
4-aminopyridine	25.0	9.82
	40.0	9.58

CHAPTER 3

KINETIC RESULTS AND DISCUSSION

Overview

Investigation of the mechanism of nucleophilic substitution of thiamin and 1'-methylthiaminium ion analogs in methanolic solution involved three types of kinetic studies: 1, nucleophilic substitution of 1'-methylthiaminium ion analogs in buffered piperidine and 4-aminopyridine solutions; 2, nucleophilic substitution of the p-nitrophenol analog of 1'-methylthiaminium ion in unbuffered solutions of 4-aminopyridine and azide ion; 3, substitution of thiamin and its pyridine analog by amine nucleophiles.

The kinetics of the substitution reactions of thiamin and its pyridine analog in methanol were investigated first. Results indicated that substitution proceeded through a unimolecular, or SN_1, type process. However, it could not be discerned whether the observed rates were affected by pH as are those of aqueous sulfite cleavage.[20] This consideration, along with the fact that the reactions studied were of long duration even at elevated temperature, resulted in a change to 1'-methylated substrates. If the reactions of thiamin in methanol were pH dependant, requiring a protonated pyrimidine ring as in the case of sulfite ion cleavage, they would be accelerated by

quaternization. This assumption proved to be correct. Preliminary NMR tube reactions revealed that substitution of 1'-methylthiaminium ion, 1b, by 4-aminopyridine took place in minutes at room temperature, rather than days at 71.5 °C required for thiamin 1a.

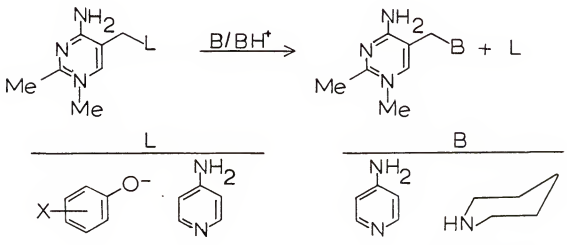
The kinetics of substitution of the p-nitrophenol analog, 7, of 1b in unbuffered 4-aminopyridine and azide ion solutions were then studied. The results, while not conclusive, indicated that the rate of substitution depended on substrate and methoxide ion concentrations, first-order in each component. Furthermore, the substitution product contained a 4-aminopyridine or azido but not a methoxy group at the methylene position. These results pointed to a mechanism much like that for aqueous sulfite ion substitution where sulfite ion serves as catalyst to generate an intermediate which is then captured by nucleophiles. The use of unbuffered solutions, however, did not allow reliable determination of methoxide ion concentration, and rate data in some cases were not reproducible.

Thus, the kinetic study of substitution of 1'-methylthiaminium ion analogs in buffered piperidine and 4-aminopyridine solutions was undertaken. The use of buffered solutions gave reproducible rate data and allowed determination of methoxide ion concentration. The kinetic and product studies confirmed the previous observations of reaction order and product composition. Since the conclusive evidence for the mechanism of substitution lies in

these studies, they will be discussed first. The results of the kinetic studies employing unbuffered solutions and those with thiamin itself will then be discussed within the context of the postulated mechanism.

Nucleophilic Substitution of 1'-Methylthiaminium Ion Analogs in Buffered Systems

The kinetics of substitution of 1'-methylthiaminium ion analogs in methanolic piperidine and 4-aminopyridine buffers were measured at 40.0±0.1 °C. Leaving groups (L) included a series of substituted phenols where the substituents were p-nitro (NMePmOØpNO$_2$, 7), p-cyano (NMePmOØpCN, 8), m-chloro (NMePmOØmCl, 9), and hydrogen (NMePmOØH, 10). In addition, one substrate having 4-aminopyridine (NMePm4AmPy, 12) as a leaving group also was examined, Equation 15. The appearance of the phenolate anion (7 through 10) and the disappearance of 12 were followed by UV spectroscopy under pseudo first-order conditions for ten half-lives in most cases.



Equation 15

In many cases kinetic runs suffered from drifting infinity values which appear to increase with time at a slow but steady rate. To compensate for unstable infinity values the pseudo first-order rate constants (k_ψ) were determined by three different methods.

The standard method calculates k_ψ from the integrated first-order rate expression, Equation 16. The infinity value, A_∞, taken at ten half-lives is itterated by computer to find the best value which fits the rate data to Equation 16[58]. Goodness of fit is determined by the correlation coefficient which ranges between 0.9996 and 0.9999 for ten to fifteen data points. The rate constant is taken from the computed best-fit. The best infinity value is usually within experimental error of that measured at ten half-lives but can be as much as 6% low for reactions followed over a period of days.

$$\ln(A_\infty - A)/(A_\infty - A_o) = -k_\psi t \qquad \text{Equation 16}$$

The Kezdy-Swinbourne method[59-62] is generally used to calculate rate constants where the infinity value is unknown and should apply to the case of drifting infinity values. The rate data are plotted according to Equation 17, where A_t is the absorbance at any time, t, and $A_{t+\tau}$ is the absorbance at a time separated from A_t by an interval, τ, which falls in the range of 1 to 1.5 half-lives. The rate constant is the antilog (base e) of the slope divided by τ, Equation 18. The point of intersection of the Kezdy-Swinbourne

line with a line of slope 1 gives the infinity value; $A_t = A_{t+\tau}$ at this point, Figure 1.

$$A_t = (A_{t+\tau})(e^{k_\psi \tau}) \qquad \text{Equation 17}$$

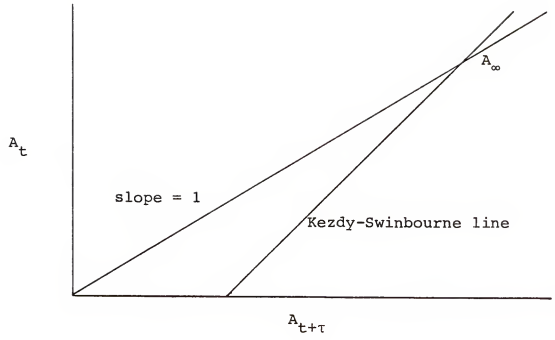
$$k_\psi = \ln \text{ slope}/\tau \qquad \text{Equation 18}$$

The third method, termed the "drift correction method," compensates for the absorbance drift by extrapolating the absorbance drift region to time zero. The difference between the extrapolated line and the absorbance curve is $A_\infty - A$, Figure 1. The rate constant is determined from a plot of $\ln(A_\infty - A)$ against time, Equation 19.

$$\ln(A_\infty - A) = -k_\psi t \qquad \text{Equation 19}$$

Pseudo first-order rate constants are tabulated in Tables 21 through 26 for at least two and usually all three methods of determination. Each method is considered equally valid as the rate constants determined by all three methods are usually within an experimental error of ±5%.

Infinity value drift is defined as the rate of increase of absorbance per hour (abs/h) for a kinetic run past ten half-lives. The major contributing factor is evaporation of the solvent from the cuvet. Using a 1 cm cuvet and teflon plug, the drift rate for a run with 10 in a 20/1 ($[B]/[BH^+]$) piperidine buffer was calculated to be 0.01 abs/h. The drift was reduced 100-fold to 0.0001 abs/h using a flame sealed cuvet. The magnitude of evaporative drift values depending on the seal of the teflon plug.



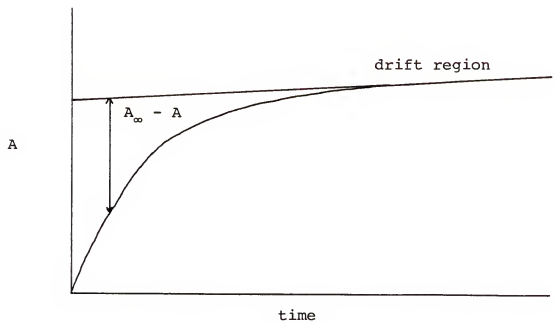


Figure 1. Graphical Representation of the Kezdy-Swinbourne and Drift Correction Methods for Infinity Drift Correction.

Many runs show little or no drift while others exhibit drifts of 0.0005 to 0.02 abs/h. Repetitive kinetic runs for 7 show drifts ranging from 0 to 0.003 abs/h, runs 4 through 9, Table 21.

Kinetic runs for 9 and 10 were performed in sealed cells to eliminate evaporation. However small the drifts were still observed. Drift rates appear to be largest for runs with high piperidine concentrations. With the buffer conjugate acid concentration fixed at 1.0×10^{-2} M, the following drift rates were observed for 9: 0.003 abs/h (40/1, $[B]/[BH^+]$), 0.0009 abs/h (10/1, $[B]/[BH^+]$), and 0.0002 abs/h (10.6/1, $[B]/[BH^+]$) at 285 nm. Similarly for 10, drift rates were observed of 0.0008 abs/h (40/1, $[B]/[BH^+]$) and 0.0001 abs/h (10/1, $[B]/[BH^+]$) at 280 nm. The buffer alone however was not measured at comparable wavelengths in a control run. In two other control runs, the piperidine substitution product and p-nitrophenol were sealed separately in cuvets with 10/1 piperidine buffers and observed at 280 and 390 nm respectively. These runs showed drifts of 0.0005 for the substitution product and 0.0004 abs/h for the phenolate anion. A 10/1 piperidine buffer did not show absorbance drift at 400 nm, the wavelength of p-nitrophenolate anion, but piperidine buffers were occasionally observed to turn yellow over a period of a few weeks. These experiments show that absorbance drift is observed for piperidine-containing solutions at widely different spectral wavelengths and is not influenced by the substitution and phenolate reaction products.

The reproducibility of individual rate constants was explored for 7, 8 and 12. Error limits are taken as ± the standard deviation of the average k_ψ determined by all methods for multiple kinetic runs. The k_ψ values for 7 show an experimental error of ±3% (Table 21, runs 5-7 and 17-18). Values for 8 do not deviate more than ±1% (Table 22, runs 4-6 and 10-12). The uncertainty in values for 12 was determined to be ±5% (Table 26, runs 1-5). Multiple measurements on 9 and 10 were not conducted due to the length of time required for each kinetic run. The maximum uncertainty in any one rate constant is assumed to be no more than ±5%.

The effect of substrate concentration on k_ψ was studied for 7. For a given set of buffer conditions the concentration of 7 was varied 10-fold, but the observed rate constants were not affected, as seen in Table 21, runs 7, 8, 10, 11 and 15-19. This observation supports the assumption of first-order dependence on substrate. Other substrates were assumed to follow this behavior.

For each substrate second-order rate constants, k_2, were calculated from pseudo first-order rate constants, k_ψ, by assuming first-order dependance on methoxide ion concentration, Equation 20. The methoxide ion concentrations for individual kinetic runs were calculated from the buffer pKa and the buffer ratio employed for a given run according to Equations 12, 13 and 14.

$$k_2 = k_\psi / [CH_3O^-] \qquad \text{Equation 20}$$

Table 21. Conditions and Rate Constants for Substitution of $NMePmO\emptyset pNO_2$ (7) in Piperidine Buffers in Methanol at 40.0 °C.

Run	$10^3 I$[b]	10^5[Substrate],M	10[B],M	10^2[BH^+],M	[B]/[BH^+]	10^5[CH_3O^-],M	$10^3 k_{\psi}, s^{-1}$[c]			$k_2, M^{-1}s^{-1}$		
							d	e	f	d	e	f
1	9.90	3.55	3.93	0.990	39.7	5.74	6.17	6.15	——	107	107	——
2	10	3.55			25[g]	3.6	4.12	——	——	114	——	——
3	10.0	3.55	2.02	1.00	20.2	2.92	3.22	3.18	——	110	109	——
4	10.4	3.55	1.00	1.04	9.62	1.39	1.59	1.58	1.56-1.60	114	114	112-115
5	10.2	3.38	1.01	1.02	9.90	1.43	1.66	1.60	1.60	116	112	112
6	10.2	3.38	1.01	1.02	9.90	1.43	1.64	1.57	1.72	115	110	120
7	10.2	3.38	1.01	1.02	9.90	1.43	1.62	——	——	113	——	——
8	10.2	0.676	1.01	1.02	9.90	1.43	1.56	1.63	1.80	109	114	126
9	10.2	3.38	0.101	0.102	9.90	1.43	1.46	1.39	——	102	97.2	——
10	10.1	0.676	0.101	1.01	1.00	0.145	——	0.153	0.156	——	106	108
11	10.1	6.76	0.101	1.01	1.00	0.145	0.154	0.159	0.169	106	110	117
12	10.1	3.38	0.101	1.01	1.00	0.145	0.163	0.167	0.178	112	115	123
13	10.2	3.38	0.101	1.02	0.990	0.143	0.167	0.166	——	117	116	——
14	10.2	3.38	0.101	1.02	0.990	0.143	0.165	0.167	——	115	117	——

Table 21. Cont'd.

Run	10^3[I][b]	10^5[Substrate],M	10[B],M	10^2[BH^+],M	[B]/[BH^+]	10^5[CH_3O^-],M	$10^3 k_\psi$,s^{-1}[c]			k_2,$M^{-1}s^{-1}$		
							d	e	f	d	e	f
15	1.01	6.76	0.0101	0.101	1.00	0.145	0.140	0.152	0.150	96.6	105	103
16	1.01	3.38	0.0101	0.101	1.00	0.145	0.164	0.161	0.179	113	111	123
17	1.02	3.38	0.0101	0.102	0.990	0.143	0.142	0.147	----	99.3	103	----
18	1.02	3.38	0.0101	0.102	0.990	0.143	0.142	0.148	----	99.3	103	----
19	1.01	0.676	0.0101	0.101	1.00	0.145	----	0.157	0.160	----	108	110
20[h]	10.0	6.76	0.0101	0.101	1.00	0.145	0.123	0.130	----	84.8	89.7	----
21[h]	10.0	3.38	0.0101	0.101	1.00	0.145	0.133	0.143	0.165	91.7	98.6	114
22[g]	10.0	0.676	0.0101	0.101	1.00	0.145	----	0.112	0.113	----	77.2	77.9
										Avg	111±7[i]	

[a] All concentrations are uncorrected for the thermal expansion of methanol.
[b] Ionic strength determinted by [BH^+].
[c] All rate constants were correlated by linear least squares regression.
[d] Standard method.
[e] Kezdy-Swinbourne method.
[f] Infinity drift correction method.
[g] Exact buffer composition is not known. Buffer ratio estimated from pH measurement.
[h] Contains $NaClO_4$ to maintain ionic strength of 1.00×10^{-2} M.
[i] Excluding runs 20, 21, and 22.

Table 22. Conditions and Rate Constants for Substitution of $NMePmOØpNO_2$[a,b] (7) in 4-Aminopyridine Buffers in Methanol at 40.0 °C.

Run	$10[B]$,M	$10^2[BH^+]$,[c]M	$[B]/[BH^+]$	$10^7[CH_3O^-]$,M	10^4k_ψ,s^{-1}[d,e]	k_2,$M^{-1}s^{-1}$	$10^2[H_2O]$,M
1	1.00	1.03	9.71	9.94	1.01	102	——
2	1.00	1.03	9.71	9.94	0.894	89.9	——
3	1.00	1.03	9.71	9.94	1.02	103	——
4	1.00	1.03	9.71	9.94	1.02	103	——
5	1.01	1.00	10.1	10.3	0.995	96.6	——
6	1.00	1.03	9.71	9.94	1.05	106	9.22
7	1.00	1.03	9.71	9.94	1.14	115	9.22
8	1.01	1.00	10.1	10.3	1.02	99.0	46.1
9[f]	0.101	0.101	10.0	10.2	1.13[e]	111	
					Avg	103±7	

[a] Substrate concentration 3.19×10^{-5} M.
[b] All concentrations are uncorrected for the thermal expansion of methanol.
[c] Ionic strength determined by $[BH^+]$ at $1.01 \pm 0.02 \times 10^{-2}$ M.
[d] Standard method.
[e] Rate constants were calculated by linear least squares regression.
[f] Ionic strength determined by $[BH^+]$ at 1.01×10^{-3} M.

Table 23. Conditions and Rate Constants for Substitution of NMePmOØpCN (8) in Methanolic Piperidine Buffers at 40.0 °C.

Run	$10[B]$, M	$10^2[BH^+]$, M[b]	$[B]/[BH^+]$	$10^5[CH_3O^-]$, M	$10^4 k_\psi$, s^{-1}[c]			k_2, $M^{-1}s^{-1}$		
					d	e	f	d	e	f
1[g]	3.93	0.990	39.7	5.74	25.0	23.9	25.8	43.6	41.6	44.9
2[g]	———	———	25[i]	3.6	16.5	16.3		46	45	
3[g]	2.02	1.00	20.2	2.92	13.5	13.4		46.2	45.9	
4[h]	1.00	1.04	9.62	1.39	6.50	6.64		46.8	47.8	
5[h]	1.01	1.02	9.90	1.43	6.62	6.70		46.3	46.9	
6[h]	1.01	1.02	9.90	1.43	6.70	6.43		46.9	45.0	
7[g]	1.01	1.02	9.90	1.43	6.50	6.34		45.5	44.3	
8[h]	1.01	1.02	9.90	1.43	6.71	6.51		46.9	45.5	
9[g]	0.500	1.02	4.90	0.708	3.20	3.29	3.34	45.2	46.5	47.2
10[h]	0.101	1.02	0.990	0.143	0.647	0.647		45.2	45.2	
11[h]	0.101	1.02	0.990	0.143	0.653	0.657		45.7	45.9	
12[h]	0.101	1.02	0.990	0.143	0.640	0.641		44.8	44.8	
							Avg	45.6±1.2		

[a] All concentrations are uncorrected for the thermal expansion of methanol.
[b] Ionic strength determined by $[BH^+]$.
[c] All rate constants were calculated by linear least squares regression.
[d] Standard method.
[e] Kezdy-Swinbourne method.
[f] Infinity drift correction method.
[g] Substrate concentration 5.88×10^{-5} M.
[h] Substrate concentration 3.32×10^{-5} M.
[i] Exact buffer composition is not known. Buffer ratio estimated from pH measurement.

Table 24. Conditions and Rate Constants for Substitution of NMePmOØmCl[a,b] (9) in Methanolic Piperidine Buffers at 40.0 °C.

Run	$10[B]$,M	$10^2[BH^+]$,M[c]	$[B]/[BH^+]$	$10^5[CH_3O^-]$,M	10^4k_ψ,s^{-1}[d]			k_2,$M^{-1}s^{-1}$		
					e	f	g	e	f	g
1	3.93	0.990	39.7	5.74	6.13	6.21	6.00	10.7	10.8	10.5
2	——	——	25[h]	3.6	3.23	3.24	3.24	9.0	9.0	9.0
3	1.08	1.02	10.6	1.53	1.03	1.04	——	6.73	6.80	——
4	1.01	1.02	9.62	1.39	1.15	1.23	1.39	7.88	8.85	10.0
5	0.500	1.02	4.90	0.708	0.668	0.665	——	9.44	9.39	——

Avg 9.12±1.3

(9.60±0.82) without run 3

[a] All concentrations are uncorrected for the thermal expansion of methanol.
[b] Substrate concentration 1.32×10^{-4} M.
[c] Rate constants were calculated by linear least squares regression.
[d] Ionic strength determined by $[BH^+]$ at $1.00 \pm 0.2 \times 10^{-2}$ M.
[e] Standard method.
[f] Kezdy-Swinbourne method.
[g] Infinity drift correction method.
[h] Exact buffer composition is not known. Buffer ratio estimated from pH measurement.

Table 25. Conditions and Rate Constants for Substitution of NMePmOØH[a] (10) in Methanolic Piperidine Buffers at 40 °C.

Run	$10^5[NMePmO\emptyset H]$,M	10[B],M	$10^2[BH^+]$,M[b]	$[B]/[BH^+]$	$10^5[CH_3O^-]$,M	$10^5 k_\psi$,s^{-1}[c]			k_2,$M^{-1}s^{-1}$		
						d	e	f	d	e	f
1	11.6	3.93	0.990	39.7	5.74		6.59	6.21-6.44		1.15	1.08-1.12
2	8.32	2.00	1.01	19.9	2.88	3.13	3.06	——	1.09	1.06	——
3	16.5	1.01	1.02	9.62	1.39	1.30	1.44	——	0.935	1.04	——
								Avg 1.07±0.07			

[a] All concentrations are uncorrected for the thermal expansion of methanol.
[b] Ionic strength determined by $[BH^+]$ at 1.00±.02 x 10^{-2} M.
[c] Rate constants were calculated by linear least squares regression.
[d] Standard method.
[e] Kezdy-Swinbourne method.
[f] Infinity drift correction method.

Table 26. Conditions and Rate Constants for Substitution of NMePm4AmPy[a,b] (12) in Methanolic Piperidine Buffers at 40.0 °C.

Run	10[B],M	$10^2[BH^+]$,M[c]	[B]/[BH$^+$]	$10^5[CH_3O^-]$,M	$10^5 k$,s^{-1} [d,e]	k_2 ,$M^{-1}s^{-1}$
1	1.01	1.02	9.90	1.43	8.84	6.18
2	1.01	1.02	9.90	1.43	10.0	6.99
3	1.01	1.02	9.90	1.43	9.29	6.49
4	1.01	1.02	9.90	1.43	8.86	6.20
5	1.01	1.02	9.90	1.43	8.84	6.18
					Avg	6.41±0.35

[a] All concentrations are uncorrected for the thermal expansion of methanol.
[b] Substrate concentration 3.36×10^{-5} M.
[c] Ionic strength determined by [BH$^+$] at 1.00×10^{-2} M.
[d] Kezdy-Swinbourne method.
[e] Rate constants were calculated by linear least squares regression.

Table 27. Summary of Second-Order Rate Constants for Methoxide Ion Catalyzed Substitution of Substrates with Phenol and Pyridine Leaving Groups at 40.0 °C.

Substituent	$k_2, M^{-1}s^{-1}$	log k_2
Phenol		
p-NO_2	107.0 ±7	2.03
p-CN	45.6 ±1.2	1.66
m-Cl	9.12±1.3	0.956
H	1.07±0.07	0.029
Pyridine		
4-NH_2	6.41±0.35	0.807

$$pH = pKa + \log [B]/[BH^+] \qquad \text{Equation 12}$$

$$pOCH_3 = pKs - pH \qquad \text{Eqaution 13}$$

$$[CH_3O^-] = \text{antilog } (-pOCH_3) \qquad \text{Equation 14}$$

Second-order rate constants were calculated for an individual run using k_ψ values as determined by the three methods previously discussed. The mean k_2 value for each substrate was determined by averaging all k_2 values for individual runs. Buffer concentrations and ratios, methoxide ion concentrations, and k_ψ and k_2 values for the reactions of 7, 8, 9, 10 and 12 at 40.0 °C are presented in Tables 21 through 26. The mean k_2 value determined for each substrate is presented in Table 27.

The experimental error for k_2 values was taken as ± one standard deviation. Substrates 7, 8, 10 and 12 show acceptable error limits, ±7, 3, 5 and 5% respectively. The error calculated for 9 was ±14%. The reason for this larger uncertainty is not known. The individual k_2 values calculated for 9 do not show trends with buffer concentration, thereby eliminating buffer catalysis as a possible explanation.

First-order dependance on methoxide ion concentration was verified for each of the phenol substrates through a log-log plot of k_ψ against the methoxide ion concentration, Figures 2 through 5, according to Equations 21 and 22. The k_ψ values for multiple runs at constant methoxide ion concentration were averaged for this determination. The kinetic order of methoxide ion, N, is the slope and the

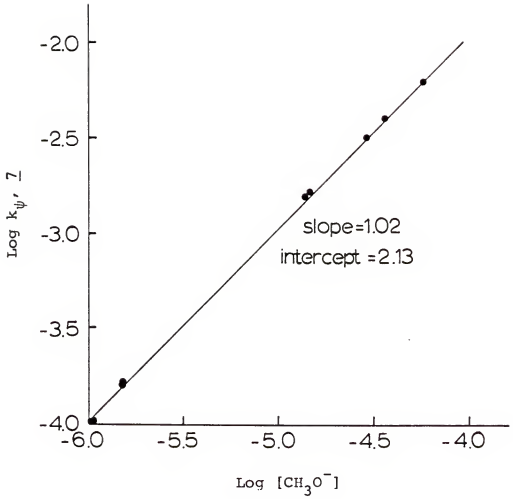


Figure 2. Log-Log Plot of k_ψ Against Methoxide Ion Concentration for $NMePmO\emptyset pNO_2$, (7).

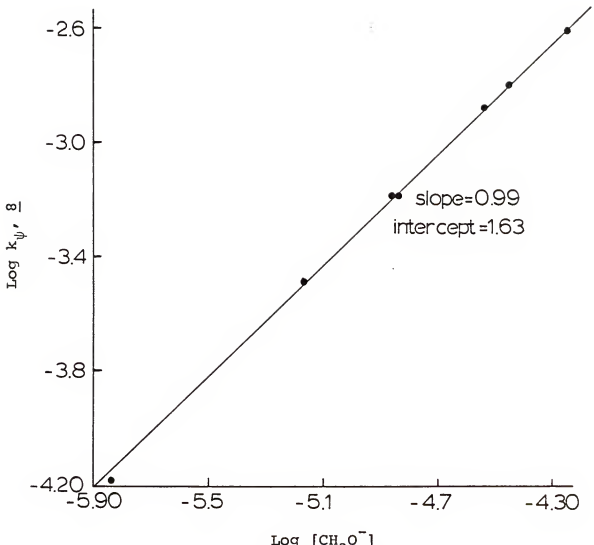


Figure 3. Log-Log Plot of k_ψ Against Methoxide Ion Concentration for NMePmOØpCN, (8).

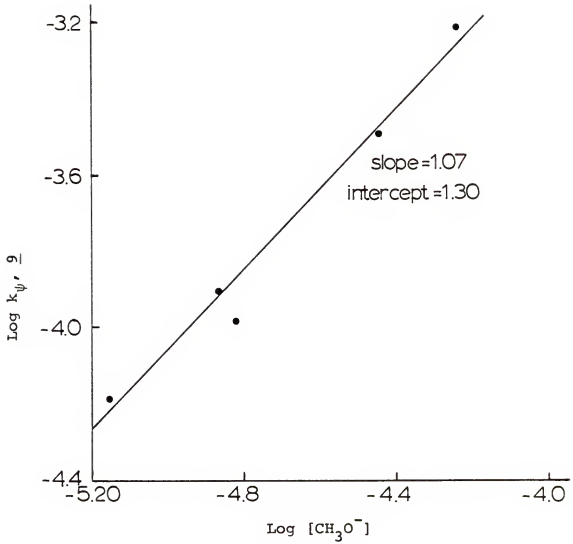


Figure 4. Log-Log Plot of k_ψ Against Methoxide Ion Concentration for NMePmOØmCl, (9).

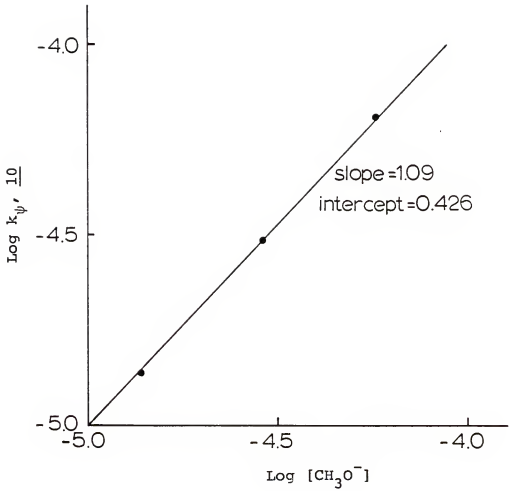


Figure 5. Log-Log Plot of k_ψ Against Methoxide Ion Concentration for NMePmOØH, (10).

second-order rate constant, k_2, is the antilog of the intercept. Results are recorded in Table 28.

$$k_\psi = k_2 [CH_3O^-]^N \qquad \text{Equation 21}$$

$$\log k_\psi = \log k_2 + N\log [CH_3O^-] \qquad \text{Equation 22}$$

The kinetic order of methoxide ion was determined to be one for each phenol substrate, slopes ranging from 0.99 to 1.09; however, the k_2 values determined by extrapolation to 1M methoxide ion concentration, the intercept of Equation 22, do not show good agreement with those determined directly from k_ψ, Equation 20. For example, the k_2 for 10 determined by extrapolation is 150% larger than the k_2 value calculated from k_ψ via Equation 20; the values are 2.67 and 1.07 $M^{-1}s^{-1}$ respectively. However, a 12% decrease in k_ψ for run 1, Table 26, brings the extrapolated k_2 value into good agreement, 1.09 $M^{-1}s^{-1}$. The slope varies only slightly, from 1.09 to 1.01. Further, a 5% decrease in k_ψ for run 1 and a 7% increase in k_ψ for run 3 also give k_2 as 1.09 $M^{-1}s^{-1}$ and a slope of 1.00. Thus small changes in k_ψ values cause large deviations in the k_2 value derived by extrapolation. Therefore k_2 values determined directly from k_ψ via Equation 20 are considered to reflect the true values.

No evidence of buffer catalysis was found for the phenol substrates with piperidine buffers. The second-order rate constant for 7 remained constant over a 400-fold change in piperidine concentration, Table 21. For example,

Table 28. Kinetic Order for Methoxide Ion and Second-Order Rate Constants for a Series of Substrates Having Phenol Leaving Groups as Determined by a Log-Log Plot of Equation 22.

Substituent	Kinetic Order[a,c]	log k_2[b,c]	$k_2, M^{-1}s^{-1}$	Corr. Coeff.	No. Pts.
p-NO_2	1.02	2.13	135	.9994	9
p-CN	0.99	1.63	42.7	.9998	7
m-Cl	1.07	1.30	20.0	.986	5
H	1.09	0.426	2.67	.9998	3

[a]Slope.
[b]Intercept.
[c]Figures 2-5.

k_2 was calculated to be 107 $M^{-1}s^{-1}$ for a 40/1 buffer, [B] = 0.393 M, run 1, and 106 $M^{-1}s^{-1}$ for a 1/1 buffer, [B] = 0.00101 M, runs 15 through 19. Similar comparisons can be made for the other phenol substrates although the variation of amine concentrations is much smaller, 40-fold for 8, 20-fold for 9 and 4-fold for 10.

The Brønsted β value measures the effectiveness of buffer catalysis. If the Brønsted β value is close to zero or one, buffer catalysis may be present but undetectable.[63] Gilbert and Jencks proposed a method to determine the minimum β value associated with lack of buffer catalysis, Equation 23, where f is the fractional increase in k_2 which could have been detected by varying the amount of buffer base.[64] Assuming the ability to detect a 15% increase in k_2, β min was

$$\beta \text{ min} = \frac{pKs - pH - \log f + \log[B]}{pKs + \log[\text{solvent}] - pKa(BH^+)} \quad \text{Equation 23}$$

calculated to be 0.65 for piperidine buffers in methanol at 40.0 °C; pKs - 16.57, $[CH_3OH]$ = 24.2 M, pKa (BH^+) = 10.73. The β min value indicates that buffer catalysis should have been detected if it were actually present. This supports the presumption that buffer catalysis is truly not present in the substitution reactions of the phenol series.

Rate studies involving the substitution of 7 in piperidine buffers were also performed at 25.0 °C. The results are given in Table 29. The second-order rate constant, 25.8 $M^{-1}s^{-1}$, determined at this temperature is a factor of 4 lower than that found at 40.0 °C, 107 $M^{-1}s^{-1}$. Using

these data, the activation parameters listed in Table 30 can be estimated from Equations 24, 25 and 26[65] where T is the temperature in °K, R is the gas constant and the factor of 4.576 is equal to 2.303R. The activation energy, Ea, is 17.6 Kcal/mole; $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are calculated to be 17 Kcal/mole and +5 cal/mole deg., respectively.

$$\log k_2 - \log k_1 = (Ea/4.576)(T_2 - T_1)/(T_2T_1) \qquad \text{Equation 24}$$

$$\Delta H^{\ddagger} = Ea - RT \qquad \text{Equation 25}$$

$$\Delta S^{\ddagger} = 4.576\ [\log k - 10.753 - \log T + Ea/4.576T] \qquad \text{Equation 26}$$

The identity of the substitution products for reactions of 7 with piperidine and 4-aminopyridine was investigated by NMR. Substitution proceeds by replacement of the p-nitrophenol group with the amine, giving the piperidine and 4-aminopyridine analogs of 1'-methylthiaminium ion. No evidence of substitution by methoxide ion was detected at the sensitivity level of proton NMR. Reactions of 8, 9, 10 and 12 are assumed to give predominantly amine substitution.

A mechanism now can be formulated. Consideration must be given to the following. The kinetic results clearly establish that substitution proceeds by a second-order process with first-order dependence on both substrate and methoxide ion concentrations; the rate expression is given by Equation 27. Furthermore, the results of product

Table 29. Conditions and Rate Constants for Substitution of NMePmOØpNO$_2$[a] (7) in Methanolic Piperidine Buffers at 25.0 °C.

Run	10[B],M	10^2[BH^+],M	[B]/[BH^+]	10^5[CH_3O^-],M	10^4k_ψ,s^{-1}[b]		k_2,$M^{-1}s^{-1}$	
					c	d	c	d
1[e]	1.08	1.02	10.6	1.33	3.62	3.65	27.1	27.3
2[f]	0.108	0.102	10.6	1.33	3.28	3.23	24.6	24.2
						Avg 25.8±1.5		

[a] Substrate concentration 3.18 x 10^{-5} M.
[b] Rate constants calculated by linear least squares regression.
[c] Standard method.
[d] Kezdy-Swinbourne method.
[e] Ionic strength determined by [BH^+] at 1.02 x 10^{-2} M.
[f] Ionic strength determined by [BH^+] at 1.02 x 10^{-3} M.

Table 30. Activation Parameters for Substitution of $NMePmO\emptyset pNO_2$ (7) in Methanolic Piperidine Buffers.

$k_2, M^{-1}s^{-1}$	log k_2	T °K	ΔEa kcal/mole	ΔH‡, kcal/mole	ΔS‡, cal/deg-mol	TΔS‡, kcal/mole	ΔG‡, kcal/mole
107	2.03	313	17.6	17.0	4.9	1.5	15.5
25.8	1.41	298		17.0	5.0	1.5	15.5

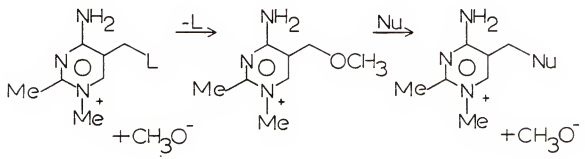
$$\text{Rate} = k_2\ [CH_3O^-][\text{substrate}] \qquad \text{Equation 27}$$

studies require separate rate and product determining steps with the formation of one or more intermediates. The minimum mechanism consistent with the data is shown in Equation 28 where the substrate, S, reacts with methoxide ion with expulsion of the leaving group, L, to give intermediate, I, in the rate determining step. The nucleophile then reacts with the intermediate with release of methoxide ion to form product P. Nucleophilic substitution directly at the

$$S + CH_3O^- \xrightarrow{-L} I \xrightarrow{Nu} P + CH_3O^- \qquad \text{Equation 28}$$

methylene carbon by an SN_1 mechanism can therefore be eliminated.

A mechanism consisting of sequential SN_2 attacks at the methylene carbon by methoxide ion followed by the product determining nucleophile, Equation 29, is not likely. To conform to observed kinetic data substitution by methoxide ion must be rate determining. However, the kinetic results



Equation 29

of the phenol series show that the leaving group ability

becomes poorer as the basicity of the leaving group increases. Therefore, methoxide ion should be a very poor leaving group and the rate determining step would be displacement of methoxide ion by the nucleophile. The kinetic expression for this mechanism would be third-order overall and contain a term for the nucleophile, Equation 30.

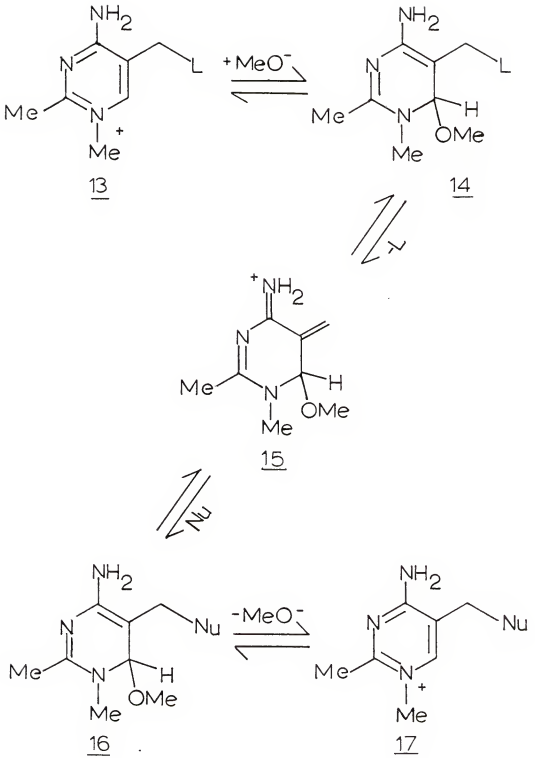
$$\text{Rate} = k\ [\text{substrate}][CH_3O^-][\text{nucleophile}]$$

Equation 30

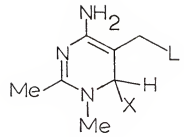
This is inconsistent with the observed kinetic data expressed by Equation 27.

A mechanism which is consistent with kinetic and product studies is postulated in Scheme 2. Rate determining formation of intermediate 15 results from a two-step process with initial attack of methoxide ion at C-6 of the pyrimidinium ring giving the pseudo base, or sigma complex 14, followed by expulsion of the leaving group. Nucleophilic capture of 15 by the buffer base and rearomatization of the pyrimidinium ring with expulsion of methoxide ion follow to give product 17.

This mechanism is supported by its close resemblance to mechanisms postulated by Zoltewicz and co-workers for sulfite and hydroxide ion catalyzed substitution reactions of thiamin and 1'-methylthiaminium derivatives in aqueous solution.[20,22-25] Both sulfite and hydroxide ions are believed to undergo initial attack at C-6 of the pyrimidinium ring to generate sigma complexes 18 and 19, comparable to the formation of 14 by methoxide ion.



Scheme 2



14 , X=OCH_3

18 , X=SO_3^-

19 , X=OH

There are numerous examples of sigma complex formation between heterocyclic compounds and sulfite[66,67] and hydroxide ions,[68-70] many of which undergo further reactions such as dehalogenation,[71] ring opening[70] and oxidation.[72] Even the 1-methylpyridinium cation for which no sigma complex can be detected in water is oxidized to the pyridone by potassium ferricyanide through the intermediacy of the hydroxy sigma complex.[72] Sigma complex formation in methanol has not been extensively studied.[70] Compounds which do form complexes with positively charged nitrogen heterocycles are the berberine cation, a derivative of isoquinoline, studied by Šimánek and co-workers[73] and the 1-phenylpyridinium cation studied by Kaválek and co-workers.[74]

The rate dependence on leaving group in the phenol series for methoxide ion catalyzed substitution of thiamin analogs is similar to that exhibited for catalysis by sulfite ion in aqueous solution. In both media a rate retardation with increasing pKa of the phenol leaving group is observed. The second-order rate constants for substitution by aqueous sulfite ion, $k_2(SO_3^=)$, are given in Table 31 and are compared to the second-order rate constants for methoxide catalyzed substitution, $k_2(MeO^-)$, from Table 27

Table 31. Rate Constants, pKa's and Hammett σ Values for Aqueous Sulfite Ion Substitution of Substrates with Phenol Leaving Groups at 25.0 °C.

Substituent	$10^3 k_2, M^{-1}s^{-1}$ [a,b]	k_2 rel	log k_2 rel	pKa[c]	σ[d]	σ⁻[d]
p-NO_2	86.4	13.6	1.13	7.15	0.81	1.24
p-CN	58.3	9.17	0.962	7.95	0.70	0.88
m-Cl	38.0	5.97	0.776	9.02	0.37	——
m-OCH_3	14.2	2.23	0.349	9.65	0.10	——
H	6.36	1.00	0.00	10.0	0	0
p-CH_3	5.98	0.940	-0.027	10.26	-0.14	——

[a] Unpublished data from work by G. Kauffman, G. Uray and J.A. Zoltewicz.
[b] Ionic strength = 1.0 M.
[c] Ref. 55; conjugate acid of the leaving group.
[d] Refs. 75,76,77.

in Figure 6. The log-log plot of $k_2(SO_3^{=})$ against $k_2(MeO^{-})$ shows a linear correlation for the p-nitro, p-cyano and m-chloro substituted substrates with only the parent phenol showing significant deviation. This deviation may indicate a change in the rate determining step. The important conclusion is that substitution in methanol may be expected to proceed by a mechanism similar to that operating for aqueous sulfite ion substitution in order to produce the linear correlation found in Figure 6. Because the sulfite ion mechanism is better established, observed similarities can be employed to develop an understanding of substitution in methanol.

The Brønsted plot correlating aqueous sulfite k_2 values with the pKa of the phenol leaving group is shown in Figure 7. The plot is non-linear showing a smaller slope for phenols with pKa's < 9, $\beta lg = -0.18$, than for phenols with pKa's > 9, $\beta lg = -0.69$. As pKa values for the appropriate phenols have not been determined in methanol, the corresponding Brønsted plot cannot be constructed for methoxide ion catalyzed reactions. However, the pKa's for phenol ionization correlate with the Hammett substituent parameter, σ^-,[75,77] and therefore comparisons of k_2 values with σ^- parameters may be made. Such a plot allows an assessment of the dependence of leaving group ability on phenol acidity. Figures 8 and 9 show a correlation of the k_2 values for aqueous sulfite and methanolic methoxide ion catalyzed substitution with the σ^- substituent parameters. As

expected from the results of the Brønsted plot, Figure 6, the Hammett plot of $k_2(SO_3^{=})$ against σ- is bimodal such that the more acidic phenols, p-nitro, p-cyano and m-chloro, fall on one line, ρ = 0.40, and the more basic phenols, m-methoxy, p-methyl and the parent phenol, fall on another, ρ = 1.7. This behavior is reproduced in the plot for methoxide ion catalyzed substitution, showing that data for p-nitro, p-cyano and m-chloro fall on a common line, ρ = 1.2, while the single point for the unsubstituted substrate deviates from this line. Evidently, the factors which operate in the aqueous sulfite ion mechanism as seen through the Hammett plot also operate in the methoxide ion mechanism to about the same extent.

The use of Hammett σ rather than σ- values results in linear correlations for both aqueous sulfite ion and methanolic methoxide systems, ρ values being 1.3 and 2.5, respectively. No bimodal character is apparent. Comparison of Figures 10 and 11 again reveals that the mechanisms must be similar.

The choice of Hammett σ or σ- values is not entirely arbitrary. The σ- scale was originally adopted in order to correlate phenol acidities. Special σ values are required for a few groups such as p-nitro and p-cyano in order to reflect the added conjugation between the phenoxide ion and these groups.

In the present context a correlation with σ- (or pKa) might be expected if loss of phenoxide ion is rate limiting

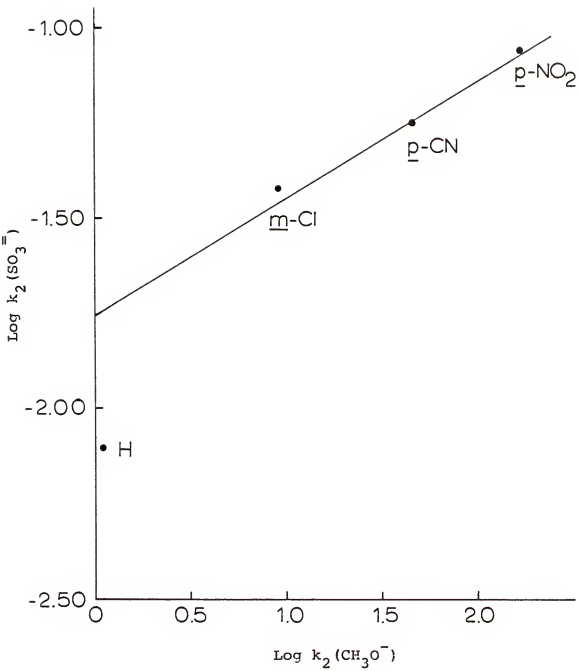


Figure 6. Correlation of Second-Order Rate Constants for Aqueous Sulfite Ion and Methanolic Methoxide Catalyzed Substitution of Phenol Analogs of 1'-Methylthiaminium Ion.

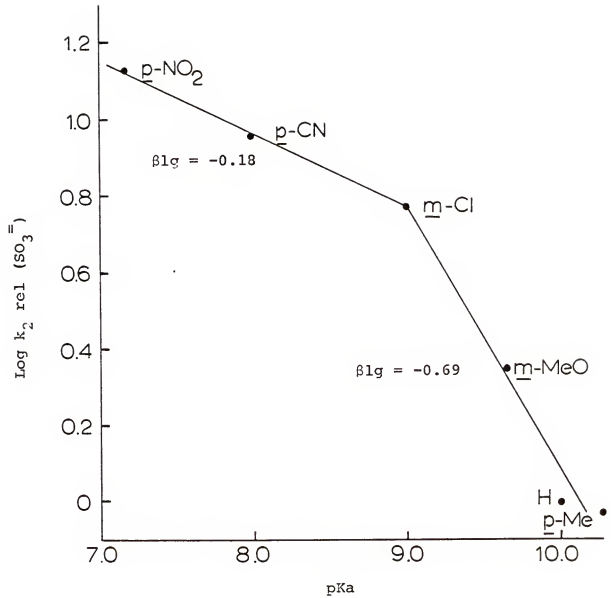


Figure 7. Brønsted Plot Correlating the Relative Second-Order Rate Constants for Aqueous Sulfite Ion Cleavage with the pKa's of the Phenol Leaving Groups.

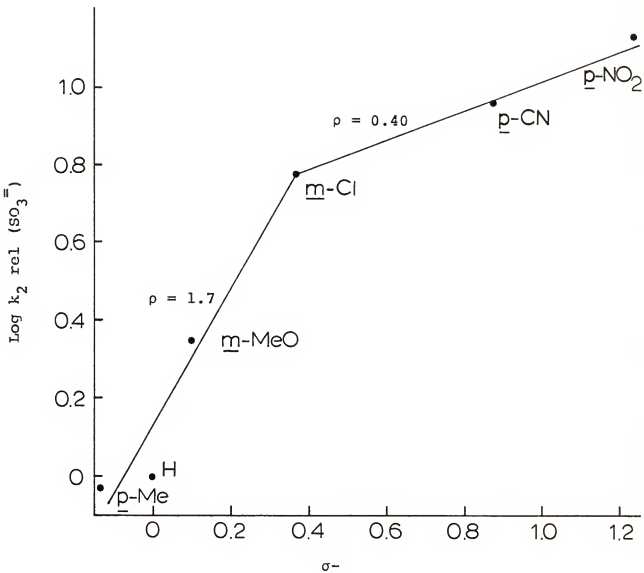


Figure 8. Hammett Plot Correlating the Relative Second-Order Rate Constants for Aqueous Sulfite Ion Cleavage with σ- Values for Phenol Leaving Groups.

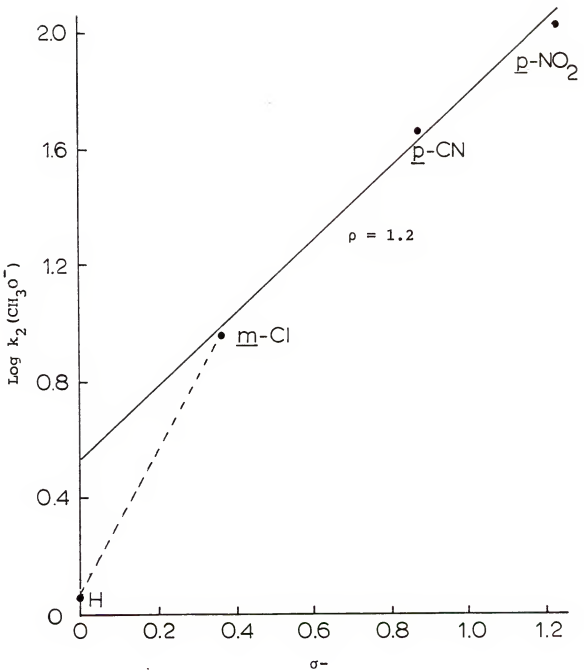


Figure 9. Hammett Plot Correlating the Second-Order Rate Constants for Methoxide Ion Catalyzed Substitution with σ- Values for Phenol Leaving Groups.

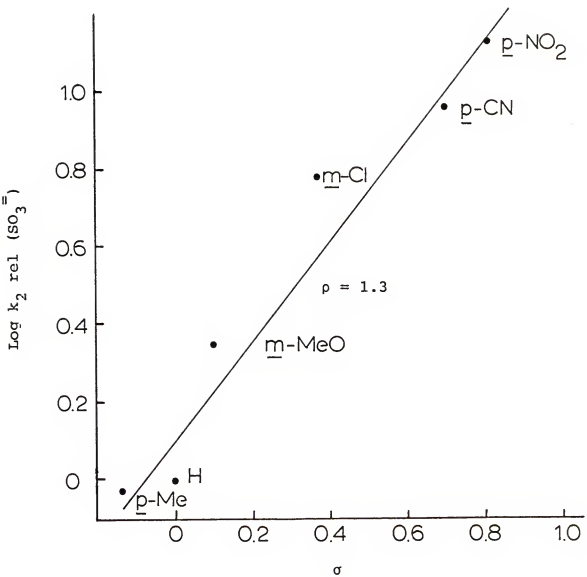


Figure 10. Hammett Plot Correlating the Relative Second-Order Rate Constants for Aqueous Sulfite Ion Cleavage with σ Values for Phenol Leaving Groups.

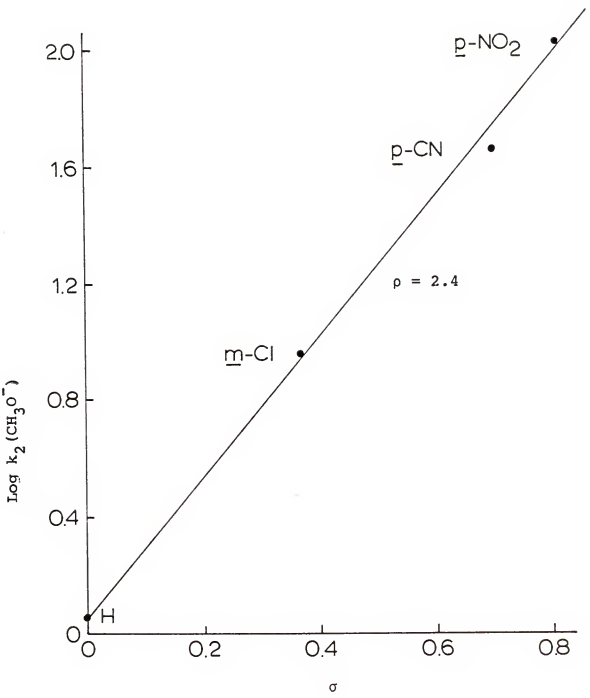


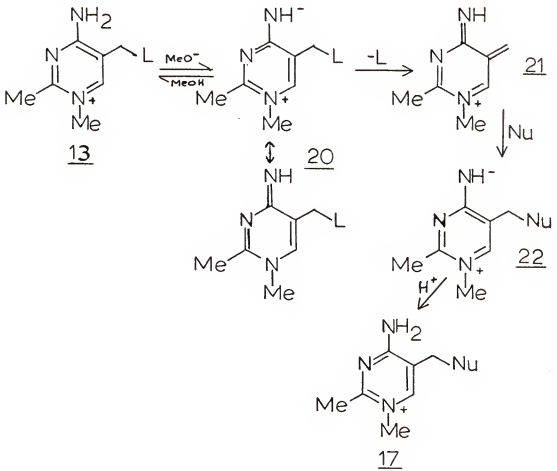
Figure 11. Hammett Plot Correlating the Second-Order Rate Constants for Methoxide Ion Catalyzed Substitution with σ Values for Phenol Leaving Groups.

and considerable charge is present on the departing anion. However, a correlation with σ might be found if addition of the nucleophile is rate limiting and the phenol only exerts an inductive effect.

Unfortunately, the Hammett σ and σ- plots do not lead to a common conclusion regarding the identity of the rate limiting step. The curved plot implies a change in the rate limiting step; the shallow slope indicates rate limiting sigma complex formation while the steep slope indicates rate limiting loss of leaving group. The linear plots, however, suggest a common rate limiting step for all substrates. At the present time no independent data exist to allow a decision to be made about which plot and hence which interpretation is to be favored. The deviation of parent phenol substrate in the correlation of $k_2(SO_3^{=})$ with $k_2(MeO^{-})$, Figure 6, however, indicates that a change in the rate determining step may occur in one or both media.

Both the linear and curved plots do allow a conclusion to be reached regarding the identity of the rate limiting step for some substrates. For those substrates having the best leaving group, both plots by their small slopes, see Figures 7 through 11, suggest a small kinetic dependence on the identity of the leaving group. In other words, the rate limiting step is not departure of the leaving group but the step prior to it: addition to the ring of the nucleophilic catalyst. No decision can be made presently about the identity of rate limiting step for the least reactive substrates.

A second mechanism can be postulated which is consistent with both rate and product studies, Scheme 3. Methoxide ion serves to deprotonate the amino group of the pyrimidinium ring forming the resonance stabilized amide ion 20; this is followed by expulsion of the leaving group to form intermediate 21. Nucleophilic capture of 21 and reprotonation give the product.



Scheme 3

Although reasonable, the mechanism is disfavored for two reasons. The close resemblance of the rate behavior for the aqueous sulfite ion cleavage to that found in methanol for methoxide ion substitution of phenol substrates argues against this mechanism. This pathway is not followed in the sulfite ion cleavage reactions. The second point is that deprotonation would be expected to be rapid with respect to expulsion of the leaving group and hence loss of the leaving group would be rate determining. Although the nature of the rate determining step remains in question for substrates with poor leaving groups such as phenol, loss of the p-nitro and p-cyano phenoxide ion leaving groups is not rate limiting. This speaks for rate determining sigma complex formation rather than amino group deprotonation.

Another possibility is that the Brønsted and Hammett plots (σ^-) do not reflect an actual change in the rate determining step but rather a change in the timing of steps for methoxide ion addition and loss of leaving group in a "concerted" SN_2' reaction. These reactions usually proceed with nucleophile and leaving group in a syn configuration.[78] This type of spatial arrangement is crowded for the substrates in this study. The syn configuration is not necessarily required, however, Yates and co-workers have stated that SN_2' reactions should require syn configuration with neutral but anti-configuration with anionic displacing groups.[79] Furthermore, Stork and Kreft[80] found the anti-configuration for intramolecular SN_2' reactions of the

thiolate anion. An SN_2' mechanism proceeding with an anti-configuration for the substitution reactions in this study could be feasible. Bordwell,[81:285] however, has stated that ". . . there appear to be no unambiguous examples of the SN_2' concerted mechanism." The SN_2' mechanism was also said to be unlikely for aqueous sulfite ion cleavage of thiamin.[20] Hence, there is insufficient evidence to support an SN_2' mechanism.

In summary, nucleophilic substitution of 1'-methylthiaminium ion analogs in methanol proceeds through a multistep mechanism in which the lyate anion serves as a catalyst. Methoxide ion attacks the C-6 position of the pyrimidinium ring to form a sigma complex followed by expulsion of the leaving group resulting in formation of an electrophilic intermediate. Nucleophiles trap the intermediate; this is followed by rearomatization of the pyrimidinium ring with loss of methoxide ion. Absence of buffer effects on the rate of reaction shows that specific base catalysis is operational. A modest leaving group effect is observed such that the rate increases with a decrease in pKa of the phenol leaving group. The exact nature of the rate determining step is in question for a few substrates. Sigma complex formation is probably rate determining for phenols more acidic than m-chlorophenol. Loss of leaving group may be rate determining for the more basic phenols but this has not been conclusively proven.

Nucleophilic Substitution of the p-Nitrophenol Analog, 7, of 1'-Methylthiaminium Ion in Unbuffered Methanolic 4-Aminopyridine and Azide Ion Solutions

The kinetics of substitution of 7 in unbuffered methanolic solutions of 4-aminopyridine and azide ion were measured at ambient temperature, 25±1 °C. The appearance of the p-nitrophenolate anion was followed by UV spectroscopy under pseudo first-order conditions. The observed rate constants, k_{ψ}, calculated as previously described, are given in Tables 32 and 33. Methoxide ion concentrations for 4-aminopyridine solutions were calculated from Equation 31 where the Kb for 4-aminopyridine is taken as 7.10, Chapter 2. The concentration of methoxide ion is a function of the square root of the 4-aminopyridine concentration.

$$[CH_3O^-] = (Kb[B])^{1/2} \qquad \text{Equation 31}$$

These kinetic studies also show a rate dependence on methoxide ion concentration. The kinetic order with respect to 4-aminopyridine and methoxide ion concentrations is graphically represented by log-log plots of k_{ψ} against the concentration of the appropriate species in Figures 12 and 13. These plots reveal the half-order dependence on 4-aminopyridine concentration and first-order dependance on methoxide ion concentration expected from Equation 31 at 4-aminopyridine concentrations greater than 4×10^{-3} M. However, Figure 12 shows that the slope of the log-log plot increases to 0.7 at 4-aminopyridine concentrationa of less than 4×10^{-3} M. Likewise, the slope of

Table 32. Conditions and Rate Constants for the Substitution of $NMePmO\emptyset pNO_2$ (7) by 4-Aminopyridine in Methanol at 25.0 °C.

Run	$10^5[NMePmO\emptyset pNO_2]$,M	[4-Aminopyridine],M	10^4k_ψ,s^{-1}			Avg. 10^4k_ψ,s^{-1}	$10^5[CH_3O^-]$,M[a]	k_2,$M^{-1}s^{-1}$	No. Half-Lives
			b	c	d				
1	5.29	1.2×10^{-1}	8.08	7.20	8.14	7.81	9.8	8.0	6.4
2	5.83	2.9×10^{-2}	3.47	4.38	4.57	4.14	4.8	8.6	5.5
3	2.01	4.0×10^{-3}	1.28	1.38	1.33	1.33	1.8	7.4	6.0
4	2.00	1.0×10^{-3}	0.473	0.453	0.470	0.465	0.89	5.2	3.1
5	2.02	2.0×10^{-4}	0.141		0.140	0.141	0.40	3.5	0.9

[a] Methoxide ion concentration calculated from Equation .
[b] Standard method.
[c] Kezdy-Swinbourne method.
[d] Guggenheim method.

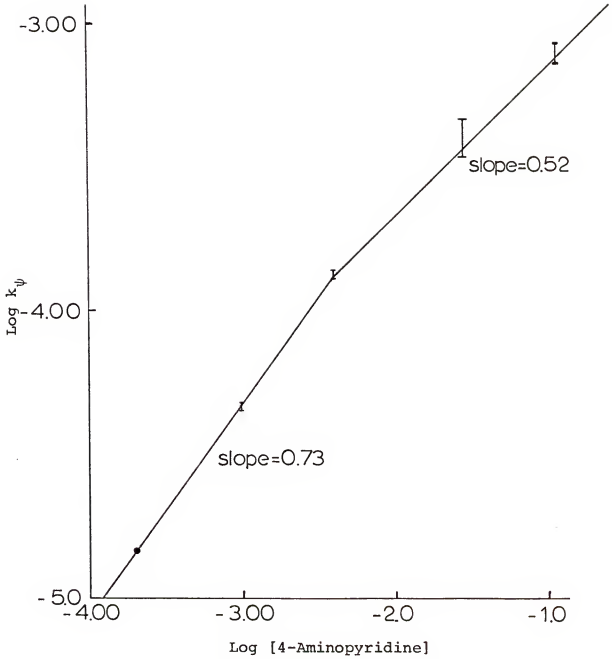


Figure 12. Log-Log Plot of k_ψ Against 4-Aminopyridine Concentration for $NMePmO\emptyset pNO_2$, (7).

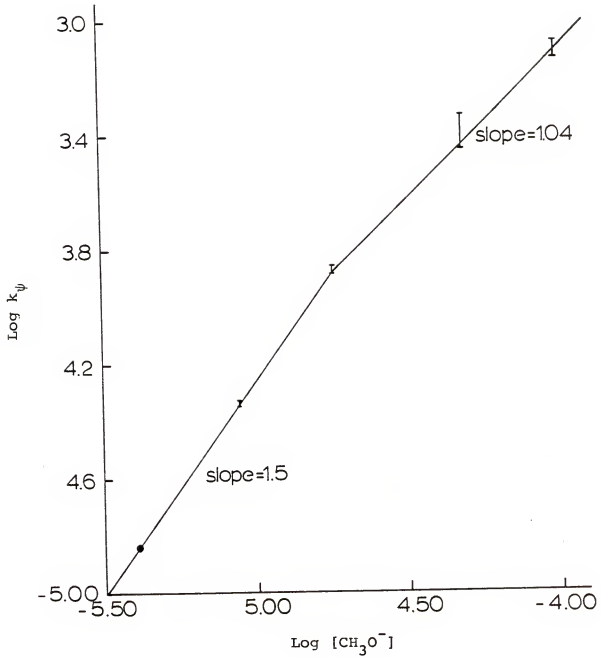


Figure 13. Log-Log Plot of k_ψ Against Methoxide Ion Concentration for $NMePmO\emptyset pNO_2$, (7).

Figure 13 increases to 1.5 at methoxide ion concentrations, calculated from Equation 31, of less than 2×10^{-5} M. This change in slope is interpreted to mean that the actual methoxide ion concentrations for these kinetic runs are less than those calculated. A kinetic determination of methoxide ion concentration can be made by extrapolation of the methoxide ion concentration, determined at high concentrations of 4-aminopyridine, to dilute solution in Figure 13. The methoxide ion concentration estimated for run 5, Table 32 ([4-aminopyridine] = 2.4×10^{-4} M) by this method is 1.7×10^{-6} M as opposed to the calculated value of 4.0×10^{-6} M. Although a 4-aminopyridine solution is not actually buffered, concentrated solutions would have more "buffer capacity," i.e., would not be as susceptible to pH changes due to CO_2 absorption and other factors as would dilute solutions.

Second-order rate constants were calculated by dividing k_ψ by the methoxide ion concentration determined by Equation 31. The average k_2 value for runs 1,2 and 3 is 8.0±0.6 $M^{-1}s^{-1}$. This value is lower than that determined with buffered piperidine solutions, 25 $M^{-1}s^{-1}$, Table 29, by a factor of 3.3. The reason for this discrepancy probably lies in the difficulty in determining the methoxide ion concentration in unbuffered solution. To equate the second-order rate constants the methoxide ion concentration of the unbuffered run 1, for example, must be decreased by a factor of 3.3 to 3.0×10^{-5} M. This figure when used to

back-calculate the pKa of 4-aminopyridine gives a value of 8.82, lower by one unit from 9.82, the value determined in Chapter 2. As the latter value correlates the k_2 value for 7 found in 4-aminopyridine buffered runs with that found in buffered piperidine runs, it is considered correct. Therefore, the k_2 values for the unbuffered runs are considered unreliable.

At high 4-aminopyridine concentrations the kinetic dependance on methoxide ion is first-order which, although not firmly established in this study, is supported by the kinetic results in buffered solutions. Furthermore, product studies revealed that the amine nucleophile and not methoxide ion is found in the final substitution product; results of the kinetic and product studies therefore follow the same form as those in buffered solutions, leading to the conclusion that the same mechanism is in operation.

The results of the azide study are far less conclusive. First-order rate constants, k_ψ, are less reproducible than those found for 4-aminopyridine. The log-log plot of k_ψ against the azide concentration shows severe curvature; low concentrations of azide ion show the expected half-order slope while higher concentrations result in a slope greater than one. The change in slope is attributed to an increase in methoxide ion concentration with increasing ionic strength.[82] These results, although not conclusive, indicate that azide ion also follows the path of the amine nucleophiles.

Table 33. Conditions and Rate Constants for the Substitution of $NMePmO\emptyset pNO_2$ (7) by Azide Ion in Methanol at 25.0°C.

Run	10^5[$NMePmO\emptyset pNO_2$],M	[NaN_3],M[a]	$10^5 k_\psi$, s^{-1}		No. Half-Lives
			b	c	
1	19.7	1.6×10^{-1}	9.55	7.49	2.7
2	6.08	1.2×10^{-1}	9.11	8.89	6.1
3	6.08	5.9×10^{-2}	3.54	3.61	3.6
4	19.7	5.4×10^{-2}	2.90	2.82	1.9
5	6.08	5.9×10^{-3}	0.90	0.89	1.0

[a]Ionic strength determined by [NaN_3].
[b]Standard method.
[c]Kezdy-Swinbourne method.

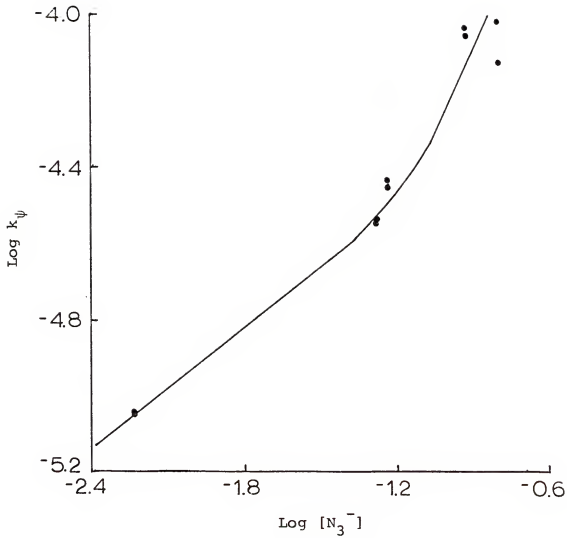


Figure 14. Log-Log Plot of k_ψ Against Azide Ion Concentration for $NMePmO\emptyset pNO_2$, (7).

Nucleophilic Substitution of Thiamin, 1a, and the Pyridine Analog, 11, by Amine Nucleophiles.

Kinetic studies of the reaction of thiamin, 1a, and its pyridine analog, 11, with amine nucleophiles pyridine, 1,4-diazabicyclo[2.2.2.]octane (Dabco), aniline, 4-aminopyridine and triethylamine were performed using methanol at 71.5 °C. Reaction progress was monitored by proton NMR over 1.5 to 4 half-lives as reported in the Experimental Section. Attempts were made to fit the kinetic data to standard first- and second-order rate equations, 32 and 33; second-order treatments assume a first-order dependence on both substrate, S, and nucleophile, N, concentrations. First- and second-order rate constants, k_ψ and k_2', and nucleophile and substrate concentrations are reported in Tables 34, 1a, and 35, 11. It should be emphasized that k_2' is obtained by fitting the rate data to the integrated second-order rate equation and is independent of k_ψ as opposed to the case of 1'-methylthiaminium ion analogs where k_2 is a function of k_ψ.

$$\ln \frac{[S]}{[N]} = -kt\,([S_o] - [N]_o) + \ln \frac{[S_o]}{[N_o]} \qquad \text{Equation 32}$$

$$\ln [S] = -kt \qquad \text{Equation 33}$$

Product identification and mass balance determination are described in the Experimental Section. Both thiamin, 1a, and the pyridine analog, 11, react quantitatively in most cases with the liberation of thiazole, 3, and pyridine, 6, respectively, and the formation of a new

Table 34. Rate Constants and Conditions for the Substitution of Thiamin (1a) by Nucleophiles in Methanol at 71.5 °C.

Run	Nucleophile	[Nucleophile], M[a]	[1a], M[a]	$10^5 k_\psi, s^{-1}$[b]	$10^5 k_2', M^{-1}s^{-1}$[c]	No. Half-lives
1[d]	pyridine-d_5	1.0	0.095	1.88	1.85	1.7
2		0.95	0.19	1.88	1.92	1.4
3		0.38	0.19	1.66	3.4-5.8 curved	2
4	4-Aminopyridine	0.95	0.19	1.47	1.89	1.4
5[d]		1.1	0.38	1.85	1.60	1.7
6		0.95	0.47	1.51	2.00	2.3
7	Aniline	1.7	0.56	1.50	1.06	3.9
8	Dabco[e]	0.95	0.47	1.84	2.19	1.5

[a]Concentrations are only good to two significant figures due to the imprecise nature of measuring solvent volume, and are corrected for the thermal expansion of methanol.
[b]First order rate constants based on concentration of thiamin.
[c]Second order rate constants based on concentration of thiamin and nucleophile.
[d]Data was taken from a small number of points and imprecise NMR signal intervals.
[e]1,4-Diazabicyclo[2.2.2]octane.

Table 35. Rate Constants and Conditions for the Substitution of 1-[(2-Methyl-4-amino-5-pyrimidinyl)methyl]pyridinium Chloride (11) by Nucleophiles in Methanol at 71.5 °C.

Run	Nucleophile	[Nucleophile], M[a]	[11], M[a]	$10^5 k_\psi, s^{-1}$[b]	$10^5 k_2', M^{-1}s^{-1}$[c]	No. Half-Lives
1	Dabco[d]	1.1	0.38	2.00	2.0	1.7
2		0.95	0.47	1.85	2.4	1.9
3	Triethylamine	0.95	0.47	1.15	1.4	1.0

[a] Concentrations are only good to two significant figures due to the imprecise nature of measuring solvent volume, and are corrected for the thermal expansion of methanol.
[b] First-order rate constants based on concentration of thiamin.
[c] Second-order rate constants based on concentration of thiamin and nucleophile.
[d] 1,4-Diazabicyclo[2.2.2]octane.

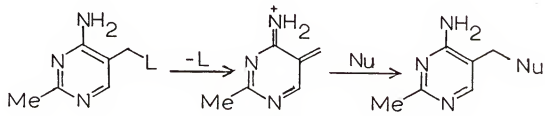
pyrimidine product. The pyrimidine product is believed to have the amine nucleophile bonded at the methylene position. Positive identification of the substitution products for the reactions of 1a with pyridine was obtained by synthesis of 11 under conditions employed for kinetic runs. The aniline adduct 23 was identified by comparison of the NMR data for the reaction product with authentic 23. That the Dabco, 4-aminopyridine and triethylamine adducts, 24, 25 and 26, were formed is based on analyses of product chemical shift data from kinetic runs and comparisons with NMR data for the 1'-methyl analogs. In no case was the product formed by methoxide ion substitution observed by NMR.

A bimolecular reaction between substrate and nucleophile can be effectively eliminated for two reasons. Inspection of Tables 34 and 35 shows that the second-order rate constants, k_2', for both substrates do not reflect the expected range of nucleophilicity exhibited by the amines employed in this study. For example, the reaction of 1a with pyridine gives k_2' as 1.9 $M^{-1}s^{-1}$, a value close to that for reaction with 4-aminopyridine, $k_2' = 1.8 \times 10^{-5}$ $M^{-1}s^{-1}$. These observations are inconsistent with a bimolecular nucleophilic substitution mechanism. Further, plots of data fitted to Equation 32 occasionally show downwards curvature after one or two half-lives. The first-order rate constants, k_ψ, for the reactions of 1a and 11 are essentially constant over the range of nucleophiles studied. For example, rate constants for the reaction of

1a with pyridine, 4-aminopyridine, aniline and Dabco are 1.9, 1.6, 1.5 and 1.8 x $10^{-5}s^{-1}$, respectively. Furthermore, the values are independent of nucleophile concentration. These observations are consistent with an SN_1-like process for which the rate expression is given by Equation 34.

$$\text{Rate} = k_{\psi}\,[\text{substrate}] \qquad \text{Equation 34}$$

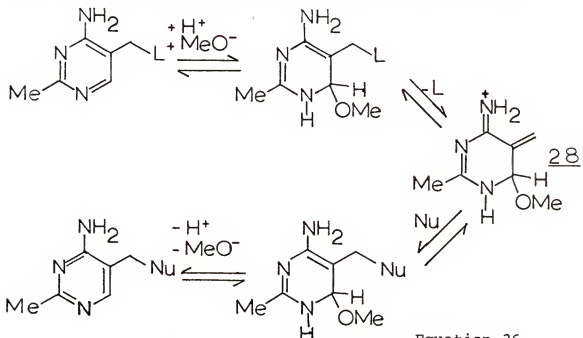
The kinetic and product studies suggest that substitution probably does not take place through an SN_1 mechanism wherein the substrate ionizes to the resonance stabilized carbonium ion, 27, in the rate determining step followed by capture of the nucleophile, Equation 35. Rate determining ionization would be expected to give rise to different rate constants since thiazole and pyridine leaving groups are different. The conjugate acids of these groups have pKa values of 3.73 and 5.22, respectively.[83,84]



27

Equation 35

In view of the mechanistic studies of aqueous sulfite[20-23, 25] and hydroxide ion[24] and methanolic methoxide ion catalyzed substitution of thiamin, 1'-methylthiamin and their analogs, it is likely that substitution proceeds according to Equation 36. The protonated substrate is converted to the sigma complex via methoxide ion attack at C-6 followed by expulsion of the leaving group in the rate determining step to form intermediate 28. Nucleophilic substitution at the methylene position and rearomatization then



Equation 36

follow. As this mechanism requires proton, methoxide ion and substrate in the rate expression, Equation 37; it is kinetically indistinguishable from Equation 36.

$$\text{Rate} = k_{\psi}\,[H^+][CH_3O^-][\text{substrate}] \quad \text{or} \quad k_{\psi}K_s\,[\text{substrate}]$$

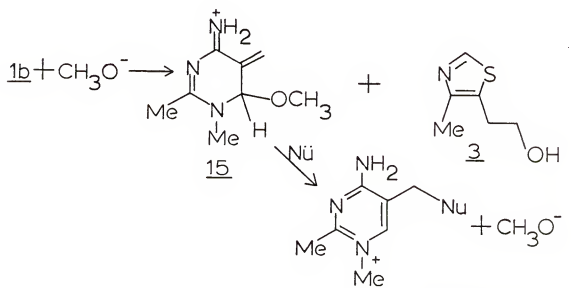
Equation 37

CHAPTER 4

SYNTHETIC ASPECTS OF NUCLEOPHILIC SUBSTITUTION OF 1'-METHYLTHIAMINIUM ION IN METHANOL

Survey of Nucleophiles

A wide variety of nucleophiles effect substitution of the 1'-methylthiaminium ion, 1b, in methanolic solution by trapping the intermediate, 15, generated from reaction of 1b with methoxide ion, Equation 38. The scope of the reaction initially investigated by Zoltewicz[85] is further extended in this work. Pyridinium and imidazolium salts; aromatic ethers, thioethers, sulfones and phosphonium salts; alkyl amines and ammonium salts; sulfonic acid and azide functionalities have been synthesized. The compounds prepared in this work are listed in Table 36 with melting points and yields.



Equation 38

Table 36. Products from the Substitution Reactions of 1'-Methylthiaminium Diperchlorate with Nucleophiles.

Nucleophile	Yield, %[a]	mp, °C[b]
phenol[c]	40	255.5-257 (dec.)
m-methoxyphenol[c]	45	289-290.5 (dec.)
m-chlorophenol[c]	85	298-299 (dec.)
p-methylphenol[c]	38	245.5-247 (dec.)
thiophenol[c]	56	157-159
2-thiopyridone[c]	77	195.5-196.5
benzenesulfinate ion[d]	31	209-210.5
sulfite ion	70	> 310
triphenylphosphine[d]	46	303-305 (dec.)
pyridine	83	262-263.5 (dec.)
2-aminopyridine	38	236-238 (dec.)
3-aminopyridine	53	207-209 (dec.)
4-aminopyridine	70	215.5-217.5
6-methyl-2-aminopyridine (exocyclic nitrogen)	90	262-263 (dec.)
Dabco	81	268-280 (dec.)
Piperidine	51	161-162.5
Azide Ion	38	114.5-116.5

[a]Yield of recrystallized product (perchlorate salts).

[b]Melting points reported for analytical samples. Microanalyses are given in the experimental section.

[c]Phenolate or thiophenolate anions were generated either with sodium hydroxide or sodium methoxide.

[d]2,4,6-Trimethylpyridine added as catalyst.

Compounds of type 17 are produced in moderate yields under relatively mild conditions. In general 1b, as the diperchlorate salt, is suspended in methanol with the nucleophile. The methoxide ion catalyst is generated *in situ* by basic nucleophiles such as amines. Phenols and thiophenols are converted to anionic nucleophiles by sodium hydroxide or methoxide. Non-basic nucleophiles such as triphenylphosphine and benzenesulfinate anion require 2,4,6-trimethylpyridine acting as a non-nucleophilic base to generate methoxide ion.

The reaction mixture is heated at reflux for 2 to 48 hours depending on the nucleophile; four hours often is sufficient. In most cases the substitution product is insoluble in methanol and is isolated directly by filtration. Soluble derivatives are isolated by solvent evaporation followed by extraction of excess nucleophile and the liberated thiazole, 3, with ethyl acetate. The remaining solid product is then isolated by filtration.

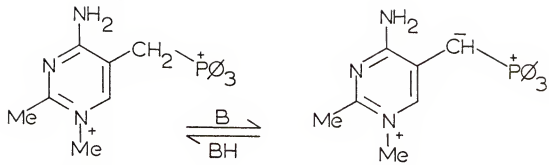
The yields reported in Table 36 were obtained after recrystallization of the crude reaction product. No attempt was made to optimize yields.

The proton NMR spectra of these compounds in Me_2SO-d_6 characteristically show two signals for the 4-amino group of the pyrimidinium ring due to restricted rotation about the C_4N bond. These signals appear between δ8.5 and 9.5 ppm. The CCH_3 and NCH_3 groups vary little in chemical shift, appearing at δ2.7 and 3.9 ppm respectively. Notably, the CCH_3 group undergoes H/D exchange when heated between

70 and 90 °C in D_2O. The pyrimidinium H-6 proton ranges in chemical shift from δ8.0 to 8.5 ppm. The factors which influence the chemical shift of this proton include shielding and deshielding effects based on the conformation of pendant aromatic groups and on inductive effects of the substituents, but these effects are not fully understood. The methylene group which bridges the pyrimidinium ring to the substituent appears as a singlet; in no instance do the two protons exhibit diastereotopic behavior. The chemical shift of the methylene group is dependent on the nature of the substituent and appears between δ4.5 and 6.0 ppm. Quaternary heterocyclic amines shift the signal of the methylene protons to low field while aliphatic amines, aromatic ethers, and thioethers shift to high field. The proton spectrum of the 4-aminopyridine derivative, 12, (Figure 15) typifies the general features present in 1-methylthiaminium ion analogs.

Significantly, the methylene group of the triphenylphosphonium derivative ($NMePmP\emptyset_3$, 29) undergoes complete H/D exchange in 10 min at 100 °C in Me_2SO-d_6/D_2O. Neither the sulfone ($NMePmSO_2\emptyset$, 30) nor the pyridinium derivatives undergo detectable exchange under similar conditions. W. von E. Doering and Hoffman obtained similar results for H/D exchange of tetramethylphosphonium and ammonium salts with deuteroxide ion.[86] It is likely that 29 exchanges as the phosphorous ylid 31, Equation 39.

The assignments for the ^{13}C spectra of the substitution products are based on off-resonance and selective proton irradiation techniques and on comparison with model



29 31

Equation 39

compounds.[87,88] The resonances of the pyrimidinium carbon atoms are fairly invarient but the methylene group exhibits a wide range of chemical shifts (24 to 64 ppm) characteristic of the heteroatom to which it is bonded. Heteroatoms produce downfield shifts in the order of O > N > S > P, Figure 16. This information can be used to determine the site of alkylation of ambident nucleophiles containing different nucleophilic heteroatoms. Thiourea and 2-thiopyridone were both shown to alkylate on sulfur rather than on nitrogen, having chemical shifts of 24.4 and 25.9 ppm respectively.

It is not yet known whether the new compounds can serve as substrates for thiaminase enzymes or inhibit thiamin's role as an enzymatic co-factor. It would be of great

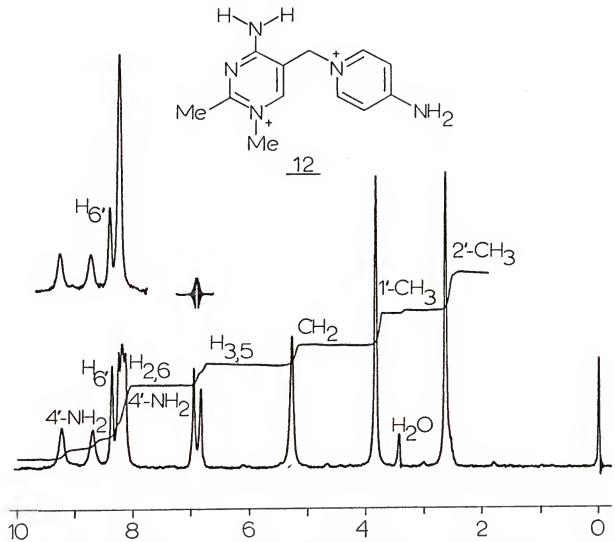


Figure 15. Proton NMR of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)methyl]-4-aminopyridinium Diperchlorate, 12, in Me_2SO-d_6. The inset shows the spin decoupling of H-3,5 used to assign H-6'.

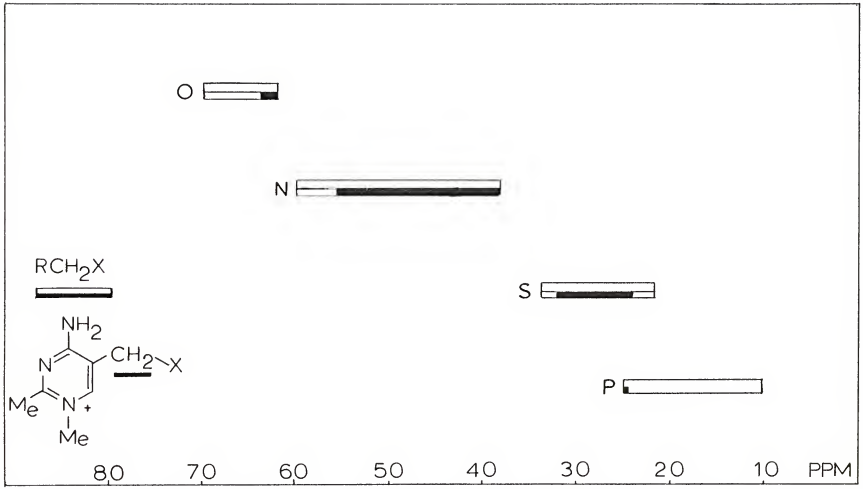


Figure 16. Carbon Chemical Shift Ranges for Methylene Groups Bonded to Heteroatoms. White blocks denote chemical shift ranges for model compounds.[87,88]

significance to find a derivative which would inhibit thiaminase activity while allowing thiamin to function in its biological role without inhibition.

Highly Fluorescent Tricyclic Compounds From 1,3-Ambident Nucleophiles.

The reactions of 1,3-ambident heterocyclic nucleophiles such as 2-aminopyridines and 2-aminothiazole with 1b represent a special class of substitution reactions. The products are bright yellow crystalline solids which emit an intense blue fluorescence in solution. Table 37 lists the nucleophiles, melting points and yields for these fluorescent compounds. Proton spectra show that the products do not contain the 4-amino group of the pyrimidinium moiety. Benzylic coupling of the pyrimidinium ring proton to the methylene group is apparent. Figures 17 and 18 show the proton spectra of the 2-aminopyridine and 2-aminothiazole substitution products; insets display the 1.5 Hz benzylic coupling. Elemental analyses confirm the loss of ammonium ion from the initial substitution product on cyclization.

Using the reaction of 1b with 2-aminopyridine as an example, a cyclization reaction of either of the potential substitution products, 32 or 33, with loss of NH_4^+ would lead to the isomeric tricyclic products, 34A or 34B, respectively, Scheme 4. Note that thiochrome[89] and pyrichrome,[90] both tricyclic, emit a blue fluorescence in solution.

Table 37. Fluorescent Cyclic Derivatives of 1'-Methylthiaminium Diperchlroate.

Nucleophile	Yield, %[a]	mp, °C[b]
2-aminopyridine	78	245-246.5 (dec.)
2-amino-3-methylpyridine	73	268.5-270 (dec.)
2-amino-4-methylpyridine	61	245-247 (dec.)
2-amino-5-bromopyridine	87	227-230 (dec.)
2-aminothiazole	45	271-273 (dec.)
thoiurea	62	290-293 (dec.)

[a]Yield of recrystallized product.

[b]Melting points reported for analytical samples. Microanalyses are given in the experimental section.

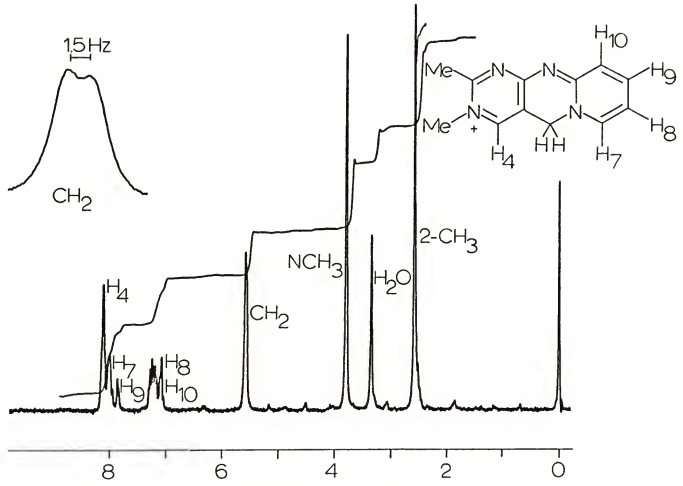


Figure 17. Proton NMR of 2,3-Dimethylpyrichrominium Perchlorate, 34. The inset shows the 1.5 Hz benzylic coupling between H-4 and the CH_2 group.

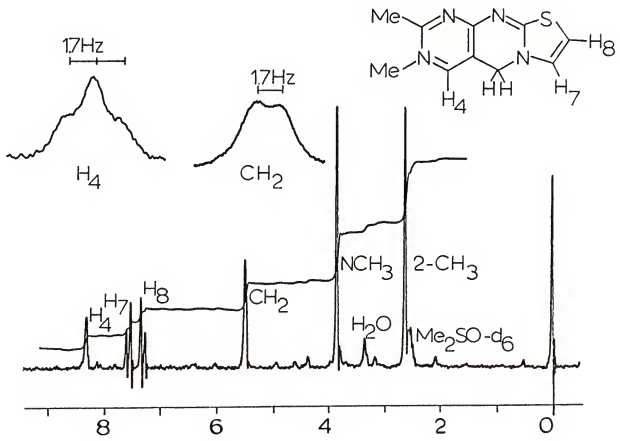
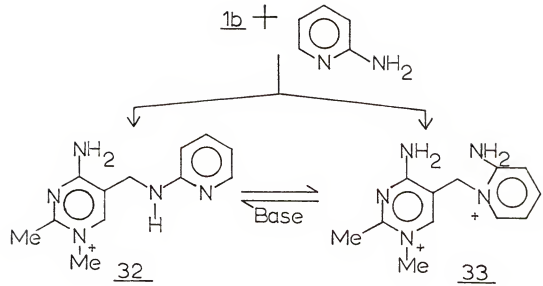
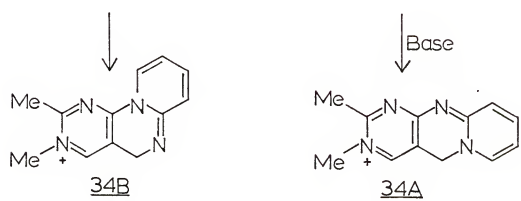
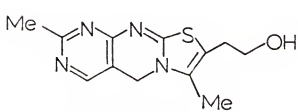


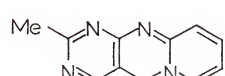
Figure 18. Proton NMR of 2,3-Dimethylthiachrominium Perchlorate, 36. The inset shows the 1.7 Hz benzylic coupling of H-4 and the CH_2 group.







thiochrome



pyrichrome

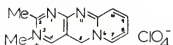
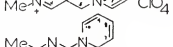
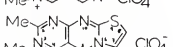
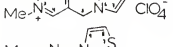
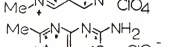
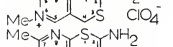
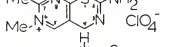
Scheme 4

Regiosomer 34A arises from alkylation of the annular nitrogen of 2-aminopyridine to give 33, followed by nucleophilic substitution where the amino group of one ring serves as a nucleophile and that of the other ring as a leaving group. Regioisomer 34B is produced by initial alkylation of the exocyclic amino group of 2-aminopyridine to give 32 followed by cyclization where the annular pyridine nitrogen displaces the 4-amino group of the pyrimidinium function.

Table 38 presents representative nomenclature and numbering for the tricyclic species discussed in this text. Compound 34A is a representative of a "linear" regioisomer, here designated "A." The three rings are fused in an anthracene configuration. Regioisomer 34B is representative of the "B" form or "bent" class of cyclic compounds with a phenanthrene configuration. With respect to trivial nomenclature, names are derived from the pyrichrome[91] or thiochrome[91] systems depending on whether a 2-aminopyridine or 2-aminothiazole is incorporated into the structure. Further, the "ium" ending is added to indicate the quaternary nitrogen. The use of the "iso" prefix as in isopyrichrominium or isothiachrominium denotes B regioisomers.

The annularly quaternized product 33 was isolated from a reaction of 1b with 2-aminopyridine at room temperature, Scheme 4. That pyridinium ion 33 was produced rather than the exocyclic alkylation product 32 is based on the pH independent NMR spectrum of the isolated compound. Chemical shifts varied little in a range of pH < 1 to 9 (see

Table 38. Representative Nomenclature for Cyclic Products.

Structure	Isomer	Systematic Name	Trivial Name
	34A	2,3-Dimethyl-5H-pyrido[1,2-a]pyrimido [4,5-d]pyrimidinium Perchlorate	2,3-Dimethylpyrichrominium Perchlorate
	34B	2,3-Dimethyl-5H-pyrido[1,2-a]pyrimido [4,5-e]pyrimidinium Perchlorate	2,3-Dimethylisopyrichrominium Perchlorate
	36A	2,3-Dimethyl-5H-thiazolo[3,2-a]pyrimido [4,5-d]pyrimidinium Perchlorate	2,3-Dimethylthiachrominium Perchlorate
	36B	2,3-Dimethyl-5H-thiazolo[3,2-a]pyrimido [5,4-e]pyrimidinium Perchlorate	2,3-Dimethylisothiachrominium Perchlorate
	43	2-Amino-6,7-dimethyl-4H-pyrimido[4,5-d]-3,1-thiazinium Perchlorate	
	45	2-Amino-6,7-dimethyl-4H-pyrimido[5,4-e]-1,3-thiazinium Perchlorate	
	46	6,7-Dimethyl-2-thio-3,4-dihydropyrimido [4,5-d]pyrimidinium Perchlorate	

Experimental Section) as expected for 33 but not for base 32. The proton chemical shifts of H-3, H-4, H-5, and H-6 of the pyridinium ring closely resemble those of the 1-methyl-2-aminopyridinium cation.[92] No CH_2-NH coupling was observed which, as will be shown, is characteristic of compounds such as 32.

The cyclization of 33 is promoted by base. Without base little cyclization occurs, as seen by the influence of amine catalysts in Table 39. Referring to Scheme 4, the base can convert one of the amino groups of 33 to the resonance-stablized amide ion, thus making it a better nucleophile in the cyclization reaction. Base also might cause isomerization of 33 to 32 via intermediate 15. Regioisomer B could conceivably arise from cyclization of 32. If this cyclization were fast, no buildup of 32 would occur.

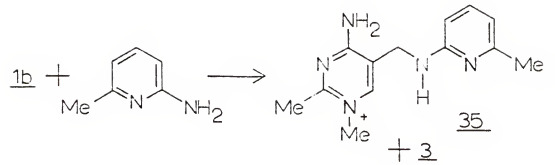
The substitution reaction of 1b with 2-amino-6-methylpyridine generates isolated product 35, Equation 40. Proof that alkylation takes place on the exocyclic amino group relies on the observation of a 7 Hz CH_2-NH coupling and pH dependent shifts of the protons bonded to the pyridine ring. A similar derivative synthesized with aniline acting as a nucleophile also shows a 7 Hz CH_2-NH coupling. The formation of the secondary amine, rather than the annularly quaternized derivative, may be due to steric crowding of the annular nitrogen. The fact that 35 does not cyclize is attributed to the same factor.

Table 39. Cyclization of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)methyl]-2-aminopyridinium Dichloride to 2,3-Dimethylpyrichrominium Chloride.

Time (h)	T, °C[a]	% Conversion		
		No Added Base	2,4,6-Collidine	Triethylamine[b]
0	25	0	0	0
1.25	25	0	0	0
2.83	75	12	54	34
17.1	75	—	100	64
113	75	16	—	—

[a]Time and temperature columns reflect the elapsed time at the specified temperature, not total time.

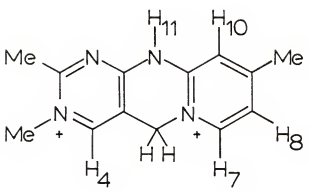
[b]At 17 h all starting material had reacted. Substitution competes with cyclization as evidenced by the production of 2-aminopyridine.



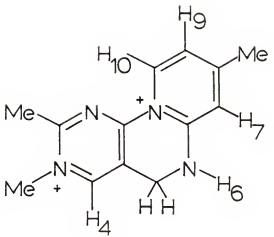
Equation 40

Proof of structure of the cyclic regioisomers was accomplished using three independent methods. The nuclear Overhauser effect (NOE) was used for the 2-aminothiazole and 4-methyl-2-aminopyridine products. X-ray analysis confirmed the findings of the 4-methyl-2-aminopyridine system. Also, the 2-aminopyridine tricyclic product was synthesized unambiguously.

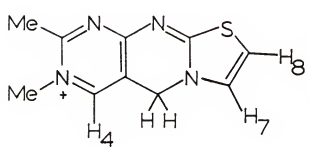
The nuclear Overhauser effect (NOE) is defined as ". . . a change in the integrated nuclear magnetic resonance (NOR) absorption intensity of a nuclear spin when the NMR absorption of another spin is saturated."[93:4] For a two spin system (A and B) in a magnetic field, H_o, the equilibrium population of the upper and lower spin states is defined by the Boltzman distribution. If the population of the two spin states for spin A is equalized by saturation with a second magnetic field, the population of spin states for spin B will redistribute itself so as to give an excess of spins in the lower spin state. Spin reorganization proceeds by a dipole-dipole spin relaxation mechanism. When the NMR



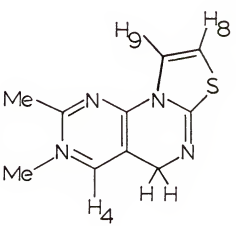
37A



37B



36A



36B

signal for spin B is measured, its intensity will be enhanced over its initial value based on the Boltzman distribution of spins. This is an NOE enhancement.

Due to the nature of the dipole-dipole relaxation mechanism, NOE enhancements depend on internuclear distances by a factor of $1/r^6$ where r is the distance between irradiated and observed nuclei. Therefore, NOE enhancements are not expected to occur between protons separated by more than about 3.7 Angstroms.[94] Interatomic proton distances approximated from Dreiding models afford a basis for judging whether or not Overhauser enhancements are likely to be observed for a particular proton with respect to a given irradiated proton. Such distances measured from Dreiding models for all protons to the methylene group of 36 and 37 are recorded in Tables 40 and 41. For 37 distances to the NH group are also included.

The results of Table 40 indicate NOE enhancements of H-4 (2.6 Å) and H-7 (3.0 Å) are likely for 36A but only for H-4 (2.7 Å) in 36B.

Similarly, Table 41 reveals that NOE enhancements are expected for protons H-4 (2.6 Å) and H-7 (2.7 Å) of 37A but only H-4 (2.6 Å) of 37B. Furthermore, distances measured with respect to the NH group predict NOE enhancements for H-10 (2.5 Å) of 37A and H-7 (2.4 Å) and the CH_2 group (2.4 Å) of 37B.

For methylene group irradiation NOE enhancements are predicted for H-4 in all structures considered for the tricyclic series. But only the A or anthracene-like isomers

Table 40. Interatomic Proton Distances for the Isomeric Forms of 2,3-Dimethylthiachrominium Perchlorate.

Isomer A		Isomer B	
Distance in Å from	to CH_2	Distance in Å from	to CH_2
2-CH_3	6.3	2-CH_3	6.2
NCH_3	4.8	NCH_3	4.7
H_4	2.6	H_4	1.7
H_7	3.0	H_9	5.8
H_8	4.9	H_8	6.7

Table 41. Interatomic Proton Distances for the Isomeric Forms of 2,3,9-Trimethylpyrichrominium Diperchlorate.

Isomer A			Isomer B		
Distance in Å from	to CH_2	to NH	Distance in Å from	to CH_2	to NH
2-CH_3	6.1	4.7	2-CH_3	6.1	> 7
9-CH_3	6.5	4.9	8-CH_3	6.9	4.8
NCH_3	4.7	6.5	NCH_3	4.7	> 7
CH_2	——	4.8	CH_2	——	2.4
H_4	2.6	5.3	H_4	2.6	4.7
H_7	2.7	5.5	H_{10}	5.6	5.5
H_8	4.7	5.8	H_9	6.8	5.9
H_{10}	5.3	2.5	H_7	4.6	2.4
NH	4.7	——	NH	2.4	——

are predicted to exhibit two NOE enhancements. While the absence of NOE enhancements cannot be taken as positive evidence for either regioisomer, the observation of two enhancements would clearly identify the A regioisomer.

The NMR spectra of the 2-aminothiazole tricyclic product, 36, showing the NOE enhancements produced on irradiation of the methylene group are reproduced in Figure 19. The control spectrum is run with irradiation at δ1.2; no signal enhancement is apparent. The inset shows the results of methylene group irradiation. The low field doublet of the AB quartet due to H-7 at δ7.7 ppm shows NOE enhancement. The H-4 proton signal of the pyrimidinium ring is also enhanced. This enhancement is due not only to an NOE but also to collapse of benzylic coupling, Figure 19, caused by irradiation of the methylene group. In these spectra signal enhancements are apparent as increases in signal intensities. The crucial observation of two enhancements provides strong evidence for the A regioisomer.

A more rigorous experiment, described in detail in the Experimental Section, was undertaken using integrated areas rather than signal intensities to determine the extent of NOE enhancements. In this experiment dilute solutions (5% wt/vol) of tricyclic compound were examined using Fourier Transform (FT) techniques. Since FT techniques require multiple data acquisitions it was necessary to determine the proton T_1 values to insure that the observed signals in NOE experiments were not saturated. The T_1

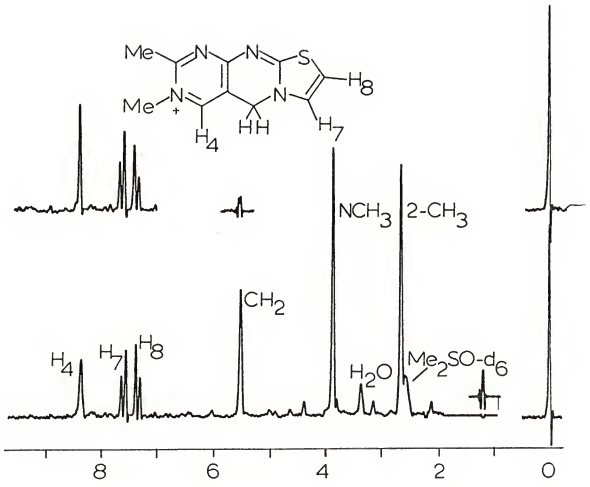


Figure 19. NOE Experiment on 2,3-Dimethylthiachrominium Perchlorate, 36. The inset shows the effect of methylene group irradiation on the intensities of H-4 and H-7.

values determined for 36 by the inversion-recovery method are listed in Table 42. To avoid saturation in FT experiments an interval of at least 5 x T_1 is needed between acquisitions. The longest T_1 value for 36 is 2.3 seconds. For NOE experiments the delay between spectral acquisitions was set at 45 second, much longer than the minimum 5 x T_1.

Table 42. T_1 Values for 2,3-Dimethylthiachrominium Perchlorate 36.

Observed Signal	2-CH_3	NCH_3	CH_2	H-4	H-7	H-8
T_1, s	0.8	0.7	0.3	0.8	1.2	2.3

Signal areas were determined by integration with respect to the methyl signal of DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate). Determination of NOE values is described in the Experimental Section. The results are given in Table 43. Two protons exhibit substantial NOE enhancements. These protons can now be positively assigned as H-4 (15% NOE) and H-7 (20% NOE) of regioisomer 36A. The negative "enhancement" noted for H-8 (-7% NOE) can be accounted for by considering the three spin system, CH_2, H-7, H-8, Figure 21. Negative "enhancements" are predicted to occur two spins away from the irradiated nucleus when the interatomic distances are such that $r_{13} > r_{12} \cong r_{23}$.[93] Estimates made from Dreiding models show that these conditions are met.

Table 43. Overhauser Enhancements for 2,3-Dimethylthiachrominium Perchlorate Derived from Methylene Group Irradiation.

	NOE (%)[a]			
Irradiate	N-3-CH_3	H_4	H_7	H_8
CH_2[b]	0	17±6	20±4	-5±10
CH_2[c]	0	14±5	20±4	-7±4
CH_2[d]	—	16±2	25±3	-10±2

[a]Error limits taken at a 99% confidence level.

[b]Integral values taken ±15 Hz from the center of the signal.

[c]Integral values taken at ±4 Hz about a signal. Values for b and c were determined using the same data set.

[d]A second sample contained a small impurity (~ 7%) appearing near H_4.

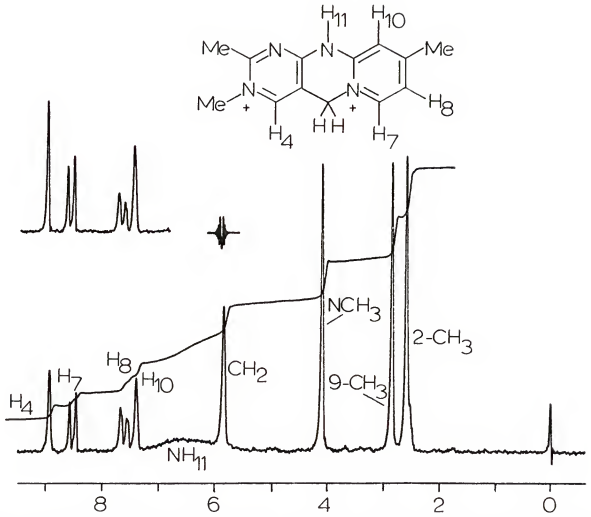


Figure 20. NOE Experiment on the Conjugate Acid of 2,3,9-Trimethylpyrichrominium Perchlorate, 37. The inset shows the effect of methylene and $N_{11}H$ group irradiation on the intensities of H-4, H-7, and H-10.

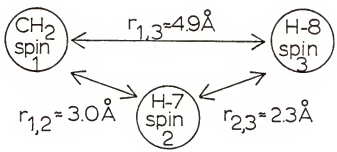


Figure 21. Interatomic Distances for the Three Spin System of 36A Estimated from Dreiding Models.

The effect of concurrent irradiation of the methylene and NH groups of 37 is shown in Figure 20. The control spectrum was irradiated at a position upfield of TMS. Particular attention is drawn to the overlap of the broad NH resonance with the methylene group. Consequently, irradiation at the CH_2 frequency also results in irradiation of NH. The inset shows signal enhancements for the pyrimidinium ring proton at δ9.0, the doublet at δ8.6 and the singlet at δ7.3. Again the enhancement at δ9.0 is due both to collapse of the benzylic spin coupling with the methylene group and to the Overhauser effect. Enhancement of the doublet at δ8.6 is indicative of isomer 37A; the proton is tentatively assigned as H-7. Enhancement of the singlet at δ7.3 may be due to an NH-H-10 Overhauser effect. Table 44 lists the Overhauser enhancements obtained from a rigorous study of integrated areas. Irradiation of the methylene group produces enhancements for H-4 (15%), H-7 (24%) and H-10 (8%) and a negative "enhancement" for the NCH_3 group (-8%). The enhancement of H-10 can be attributed to NH-H-10 Overhauser interaction because selective

Table 44. Overhauser Enhancements for 2,3,9-Trimethylpyrichrominium Diperchlorate Derived from Methylene and $N_{11}H$ Group Interaction.

	NOE (%)[a]							
Irradiate	$9\text{-}CH_3$	$2\text{-}CH_3$	N_3CH_3	CH_2	H_4	H_7	H_8	H_{10}
CH_2[b]	0	0	-8±4	—	15±4	24±6	0	8±5
NH[b]	0	0	-10±6	-9±5	0	0	0	13±4

[a]Error limits taken at 99% confidence level.

[b]Due to the slight overlap of the broad NH and the CH_2 signals, irradiation of the CH_2 group causes enhancements of signals close to the NH group, i.e., H_{10}.

irradiation of just the NH signal produces enhancements of 13% in H-10 and 0% in H-4 and H-7, but -9% and -10% decreases in the methylene and NCH_3 groups respectively. The simple linear three spin analogy for negative "enhancements" fails to account for the methylene and NCH_3 group area decreases. However, this spin system is notably more complex. These experiments clearly identify the regioisomer as 37A and N-11 as the site of protonation.

The structure of 37 was further confirmed by x-ray analysis.[95] X-ray data show the solid state structure to be the A regioisomer. Bond angle and bond length data are given in Table 45.

A third confirmation of structure was accomplished by the unambiguous synthesis of the A regioisomer of 2,3-dimethylpyrichrominium perchlorate, 34. Substitution of 1b with 2-methoxypyridine resulted in the formation of 34A. The site of alkylation of 2-methoxypyridine is unambiguous. No possibilities exist for base-catalyzed isomerization, and cyclization must proceed by nucleophilic displacement of the methoxy group by the 4-amino group of the pyrimidinium ring, Equation 41. Melting point and spectral data are identical to those of 34 synthesized from 1b and 2-aminopyridine.

The reactions of 2-amino heterocycles give rise to "A" cyclic products. The reaction of 1b with the biological base, adenosine, also gives a tetracyclic "A" compound. Work with adenosine and adenine was published previously and is reprinted in the Appendix.

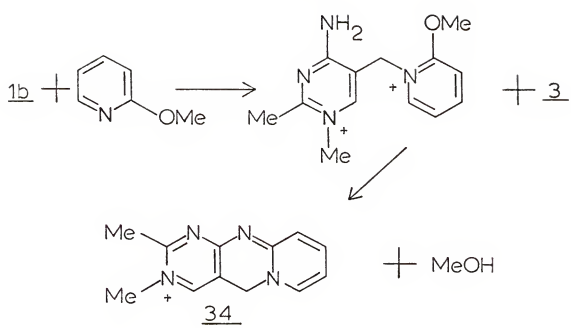
Table 45. Bond Lengths and Angles in 2,3,9-Trimethylpyrichrominium Diperchlorate by X-ray Analysis.[a]

Atoms	Distance (Å)	Atoms	Angles (degrees)
N1-C2	1.289	N1-C2-CH_3	119.5
C2-CH_3	1.545	N1-C2-N3	122.5
C2-N3	1.330	CH_3-C2-N3	118.0
N3-CH_3	1.538	C2-N3-C4	118.8
N3-C4	1.328	C2-N3-CH_3	121.1
C4-C4a	1.322	CH_3-N3-C4	119.9
C4a-C11a	1.406	N3-C4-C4a	123.0
C11a-N1	1.323	C4-C4a-C11a	115.4
C4a-C5	1.485	C4a-C11a-N1	121.3
C5-N6	1.491	C4-C4a-C5	125.8
N6-C10a	1.324	C11a-C4a-C5	118.8
C10a-N11	1.357	C4a-C5-N6	116.3
N11-C11a	1.332	C5-N6-C10a	121.3
N6-C7	1.369	N6-C10a-N11	121.1
C7-C8	1.319	C10a-N11-C11a	122.2
C8-C9	1.412	N11-C11a-C4a	120.3
C9-CH_3	1.506	N11-C11a-N1	118.4
C9-C10	1.376	C5-N6-C7	117.9
C10-C10a	1.396	N6-C7-C8	121.6
		C7-C8-C9	120.7
		C8-C9-CH_3	116.5
		C8-C8-CH_3	120.8
		CH_3-C9-C10	122.7

Table 45. Continued.

Atoms	Distance (Å)	Atoms	Angles (degrees)
		C9-C10-C10a	121.5
		C10-C10a-N6	119.0
		C10a-N6-C7	120.7

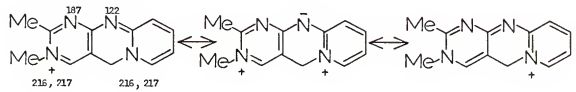
[a]Data supplied by the courtesy of A. W. Cordes, University of Arkansas.

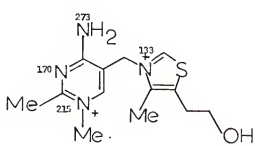


34

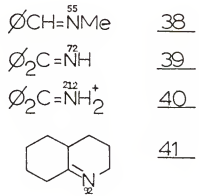
The fluorescence properties of the compounds listed in Table 37 are quite remarkable. Concentrated solutions show little fluorescence primarily due to self-quenching; however dilute solutions emit a blue fluorescence even when excited by weak sources such as sunlight or room lights. The fluorescence quantum yield of 37 was determined by a group in Poland[96] to be 0.80 , close to the limit of unity. The pyrichrominium compounds all fluoresce in the region of 420 and 460 nm, with the band at longer wavelength appearing as a shoulder. A bromine substituent in the 8 position of 34 shifts the fluorescence to a slightly longer wavelength, 448 nm, with a shoulder at 470 nm. The thiachrominium compound fluoresces at 452 nm. The excitation wavelength for all species falls at about 400 nm. Notably, excitation of the conjugate acid of 37 (350 nm) still results in fluorescence emission from the base, showing the excited state to be more acidic than the ground state.

The ^{15}N NMR spectrum of 34 shows four resonances appearing at 122, 187, 216 and 217 ppm. The chemical shift of the nitrogen atom in nitromethane is taken as a reference. Positive shifts are located upfield to the standard. The previously determined nitrogen shifts of the pyrimidinium ring of 1b can be used by analogy to assign N-1 and N-3 of 34.[97] The quaternary nitrogen, N-3, is assigned to one of the highfield resonances, 216 or 217 ppm, while ring nitrogen N-1 appears at lower field, 187 ppm. Consideration of the resonance forms shown below reveals considerable positive charge should also be located at N-6 which therefore must appear as the other highfield resonance, 216 or 217 ppm. The remaining resonance at 122 ppm is assigned to the imine-like N-11. Model compounds (38-41) with imine-like nitrogens typically resonate at low field.[98,99]



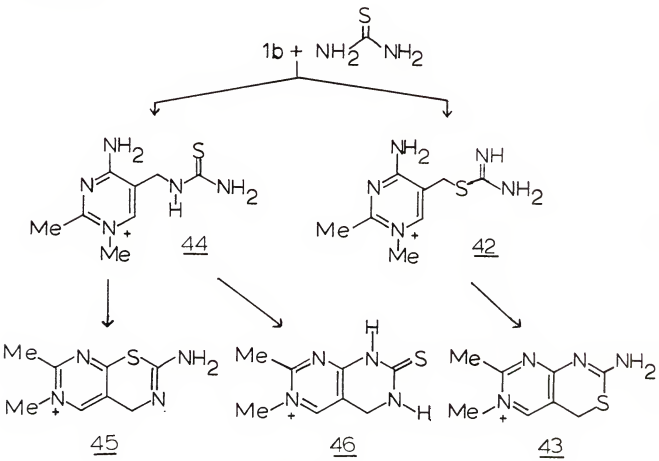


1b



Thiourea reacts with 1b in methanol. Elemental analysis reveals loss of an amino group from the reactants. A cyclization reaction producing a bicyclic product is therefore probable.

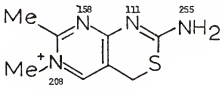
The structures of three possible bicyclic compounds are shown in Scheme 5. Initial substitution with alkylation of thiourea on sulfur would produce the isothiourea 42 which could cyclize to the pyrimidothiazine 43. If the initial substitution proceeds via nitrogen alkylation, then intermediate thiourea 44 would be produced which could cyclize to the pyrimidothiazine 45 or the pyrimidopyrimidine 46.



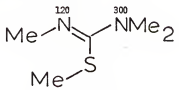
Scheme 5

Carbon 13 NMR clearly identifies 43 as the product. The crucial observation is the chemical shift of the methylene group at 24.4 ppm. With reference to Figure 15 this chemical shift is consistent only with the methylene group adjacent to sulfur, thereby eliminating structures 45 and 46.

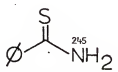
Nitrogen 15 chemical shifts also confirm structure 43; they are observed at 111.3, 157.8, 209.1 and 255.0 ppm upfield of nitromethane. The resonances at 157.8 and 208.1 ppm are assigned to N-6 and N-8 of the pyrimidinium ring by analogy to the pyrimidinium ring of 1b.[97] The signal at 111.3 is assigned to N-1 by comparison with isothiourea 47.[98] The remaining signal at 255 ppm is assigned to the amino group. The amino groups of model compounds 1b and 47 through 49 fall in the range of 245 to 300 ppm. Interestingly, the triplet expected for the amino group does not appear in the proton coupled spectrum; the signal is broadened into the baseline. However, the amino signal appears as a fairly sharp singlet in the proton decoupled spectrum.



43



47



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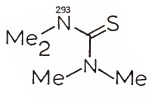
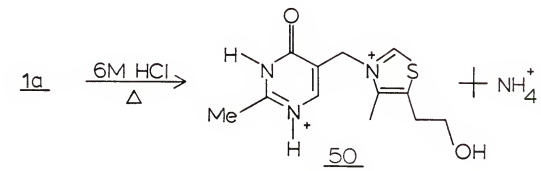


49

Bicyclic product 43 also exhibits fluorescence properties. The wavelengths for excitation and emission are 328 and 390 nm, respectively. The fluorescence is therefore in the ultraviolet range.

Syntheses of 1'-Methyloxythiamin Derivatives

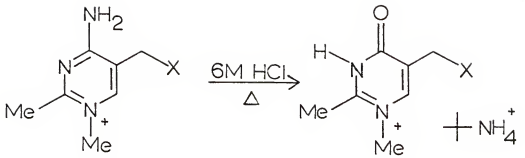
Oxythiamin, 50, is a noted antagonist, or antivitamin, of vitamin B_1.[30] Heating thiamin in refluxing hydrochloric acid gives oxythiamin, Equation 42.



50

Equation 42

A convenient synthesis of the hitherto unknown 1'-methyloxythiamin compounds was developed by heating 1'-methylthiaminium ion analogs in 6M HCl. In this manner 1'-methyloxythiamin analogs were prepared where X is thiazole 3, pyridine, N-methylimidazole and thiophenol, Equation 43. Yields and melting points of these derivatives are recorded in Table 46.



Equation 43

Attempts to produce a phenol derivative according to Equation 43 failed. The attempted conversion of the p-methylphenol derivative resulted in complete ether cleavage. One of the products was identified by NMR as p-methylphenol; the other was a pyrimidine or pyrimidone of unidentified structure.

These compounds may find application as substrates for thiamin and thiaminase enzymes. Their behaviour in substitution reactions analogous to those of the 1'-methylthiaminium ion has yet to be explored.

Table 46. Substituted 4-Pyrimidone Compounds Derived from 1'-Methylated Thiamin Analogs.

Substituent	Yield, %[a]	mp, °C[b]
4-(2-hydroxyethyl)-5-methylthiazole	60	164.5-166
pyridine	47	265-266.5 (dec.)
3-methylimidazole	32	203-205 (dec.)
thiophenol	89	162-163.5

[a]Yield of recrystallized product.

[b]Melting points reported for analytical samples. Microanalyses are given in the Experimental Section.

CHAPTER 5

EXPERIMENTAL

Instrumentation

Proton, carbon, and nitrogen magnetic resonance spectra were obtained on the following instruments: Varian Associates A-60A, EM 360-L, XL-100-15; Jeol PMX-60, FX-100; Nicolet-NT-300. Ultraviolet and visible spectra were recorded on a Cary 17-D or Perkin-Elmer 330 spectrophotometer. Fluorescence spectra were obtained on a Perkin-Elmer 153 spectrophotometer and are uncorrected. Measurements of pH were determined with a Radiometer PHM 64 pH Meter equipped with Radiometer GK 2321C or GK 2401B combination electrodes. Temperature control for kinetic runs and pKa determinations was maintained with a Lauda-Brinkman K-2/R circulating temperature controller. Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. Chemical analyses were performed by Atlantic Microlabs.

Chemicals

All common laboratory chemicals, unless otherwise specified, were reagent grade and from various suppliers. Deuterium oxide (99.5 atom percent) was supplied by Columbia Organic Chemicals. Dimethyl sulfoxide-d_6 (99.5 atom percent) was supplied by Aldrich Chemicals. 1'-Methylthiaminium

diperchlorate was synthesized by the method of Zoltewicz and Baugh.[97]

Preparations

Preparation of 4-Amino-1,2-dimethyl-5-phenoxymethylpyrimidinium Perchlorate (10). To a suspension of 1'-methylthiaminium diperchlorate (2.52 g, 5.28 mmoL) and phenol (1.17 g, 12.5 mmoL) in methanol (30 mL) was added methanolic sodium hydroxide (0.211 g, 5.28 mmoL in 2.57 mL). Following heating at reflux for 8 h and ice cooling for 30 min, the mixture was filtered. The crude product was washed first with ethyl acetate (2 x 5 mL) then with ethyl ether (2 x 5 mL) to give 1.10 g of product, mp 247-250 °C (dec.). Recrystallization from a 1:3 (v/v) mixture of dimethylformamide and 0.1 M perchloric acid gave 0.70 g (2.12 mmoL, 40%) of a white solid, mp 252-255 °C (dec.). An analytical sample was prepared by further recrystallization from 20% aqueous dimethylformamide follwed by vacuum drying at 100 °C over magnesium perchlorate for 2 h, mp 255.5-257 °C (dec.): ^{1}H NMR (c. 0.76 M, Me_2SO-d_6, Me_4Si) δ2.59 (2-CH_3), 3.82 (NCH_3), 4.94 (CH_2), 6.9-7.4 (aromatic mult.), 8.43 (H_6), 8.51, 9.10 (NH_2); ^{13}C NMR (c. 0.76 M, Me_2SO-d_6, Me_4Si) δ21.5 (2-CH_3), 41.7 (NCH_3), 62.3 (CH_2), 111.6 (C_5), 114.7 (C_2'), 121.3 (C_4'), 129.5 (C_3'), 146.8 (C_6), 157.6 (C_1'), 161.6, 162.2 (C_2, C_4).

Anal. Calcd for $C_{13}H_{10}ClN_3O_5$ (329.7): C, 47.35; H, 4.89; N, 12.74. Found: C, 47.45; H, 4.92; N, 12.76.

Preparation of 4-Amino-1,2-dimethyl-5-[3-methoxyphenoxy]-methylpyrimidinium Perchlorate (51). To a suspension of

1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) and 3-methoxyphenol (3.00 g, 24.2 mmoL) in methanol (50 mL) heated to reflux was added methanolic sodium hydroxide (0.516 g, 12.9 mmoL in 3.00 mL). Heating was continued for 2 h followed by evaporation of the solvent under reduced pressure. The residue was triturated with ethyl acetate (20 mL); the resulting solid was filtered and washed first with ethyl acetate (3 x 10 mL) then acetone (10 mL), giving 2.37 g of a slightly yellow solid, mp 270-275 °C (dec.). Recrystallization from a 2:3 (v/v) mixture of dimethylformamide and 0.1 M perchloric acid gave 1.68 g (4.67 mmoL, 45%) of a white solid, mp 286-288 °C (dec.). A sample was prepared for analysis by three further recrystallizations from 50% aqueous dimethylformamide followed by vacuum drying at 100 °C over magnesium perchlorate for 12 h, mp 289-290.5 °C (dec.): ^{1}H NMR (c.0.70 M, Me_2SO-d_6, 80 °C, Me_4Si) δ2.60 (2-CH_3), 3.76 (OCH_3), 3.83 (NCH_3), 4.97 (CH_2), 6.5-7.3 (aromatic mult.), 8.36 (H_6), 8.57 (NH_2, broad); ^{13}C NMR (c. 0.70 M, Me_2SO-d_6, 80 °C, Me_4Si) δ21.5 (2-CH_3), 41.9 (NCH_3), 55.4 (OCH_3), 62.9 (CH_2), 101.8 (C_2'), 107.5 (C_4'), 112.0 (C_5), 130.0 (C_5'), 146.8 (C_6), 159.0, 160.8 (C_1', C_3'), 161.7, 162.2 (C_2, C_4).

Anal. Calcd for $C_{14}H_{18}ClN_3O_6$ (359.8): C, 46.74; H, 5.04; N, 11.68. Found: C, 46.78; H, 5.04; N, 11.69.

Preparation of 4-Amino-1,2-dimethyl-5-[3-chlorophenoxy]-methylpyrimidinium Perchlorate (9). 3-Chlorophenol (4.01 g, 31.2 mmoL) in methanol (10 mL) partially converted to the phenolate by addition of sodium methoxide (7.00 mL of 1.67 M,

11.7 mmoL) was added by drops to a stirred suspension of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) in methanol (50 mL). The suspension was heated at reflux for 8 h followed by ice cooling (30 min) and filtration. The resulting solid was washed first with ice-cold methanol (2 x 10 mL) then ethyl acetate (2 x 10 mL) and finally ethyl ether (2 x 10 mL) to give 3.40 g of product, mp 285-287 °C (dec.). Recrystallization from a 1:1 (v/v) mixture of dimethylformamide and 0.1 M perchloric acid yielded 3.20 g (8.79 mmoL, 85%) of product, mp 297-300 °C (dec.). An analytical sample was prepared by further recrystallization from 50% aqueous dimethylformamide followed by heating a suspension of the compound in ethanol at 45 °C for 30 min then vacuum drying at 100 °C over magnesium perchlorate for 3 h, mp 298-299 °C (dec.): ^{1}H NMR (c. 0.70 M, Me_2SO-d_6, 45 °C, Me_4Si) δ2.61 (2-CH_3), 3.83 (NCH_3), 4.98 (CH_2), 7.01-7.46 (aromatic mult.), 8.43 (H_6), 8.43, 9.06 (NH_2); ^{13}C NMR (c. 0.70 M, Me_2SO-d_6, 45 °C, Me_4Si) δ21.6 (2-CH_3), 41.8 (NCH_3), 62.9 (CH_2), 111.3 (C_5), 113.9 (C_6'), 114.9 (C_2'), 121.3 (C_4'), 130.9 (C_5'), 133.8 (C_3'), 147.0 (C_6), 158.6 (C_1'), 161.6, 162.3 (C_2, C_4).

Anal. Calcd for $C_{13}H_{15}Cl_2N_3O_5$ (364.2): C, 42.87; H, 4.15; N, 11.54. Found: C, 42.86; H, 4.16; N, 11.53.

Preparation of 4-Amino-1,2-dimethyl-5-[4-methylphenoxy]-methylpyrimidinium Perchlorate (52). 1'-Methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) was suspended in a solution of 4-methylphenol (3.00 g, 27.7 mmoL) in methanol (50 mL) and heated to reflux. Methanolic sodium hydroxide

(0.516 g, 12.9 mmoL in 3.00 mL) was added to the suspension and heating continued for 2 h. The solvent was removed under reduced pressure and the residue triturated with ethyl acetate (20 mL). The resulting solid was washed first with ethyl acetate (3 x 10 mL) then acetone to give 1.68 g of a light tan material, mp 235-245 °C (dec.). Recrystallization from 20% aqueous dimethylformamide and charcoal produced 1.35 g (4.01 mmoL, 38%) of slightly off-white needles, mp 243-245 °C (dec.). An analytical sample was prepared by three further recrystallizations from 25% aqueous dimethylformamide resulting in white crystals which were vacuum dried at 100 °C over magnesium perchlorate, mp 245.5-247 °C (dec.): ^{1}H NMR (c. 0.70 M, Me_2SO-d_6, Me_4Si) δ2.25 (4'-CH_3), 2.59 (2-CH_3), 3.82 (NCH_3), 4.90 (CH_2), 6.95 (H_2', H_6', apparent AB, $J_{2',3'}$ ≅ 8 Hz), 7.15 (H_3', H_5', apparent AB, $J_{2',3'}$ ≅ 8 Hz), 8.40 (H_6), 8.47, 9.08 (NH_2); ^{13}C NMR (c. 0.70 M, Me_2SO-d_6, Me_4Si) δ20.0 (4'-CH_3), 21.5 (2-CH_3), 41.7 (NCH_3), 62.5 (CH_2), 111.8 (C_5), 114.6 (C_2'), 129.8 (C_3'), 130.2 (C_4'), 146.6 (C_6), 155.5 (C_1'), 161.5, 162.2 (C_2, C_4).

Anal. Calcd for $C_{14}H_{18}ClN_3O_5$ (343.8): C, 48.91; H, 5.28; N, 12.22. Found: C, 48.95; H, 5.29; N, 12.22.

Preparation of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)-methyl]pyridinium Diperchlorate (53). A suspension of 1'-methylthiaminium diperchlorate (4.79 g, 10.0 mmoL) and pyridine (2.53 g, 32.0 mmoL) in methanol (50 mL) was heated at reflux for 48 h. The resulting mixture was cooled in ice for 30 min and filtered. The crude material was washed

with methanol (2 x 5 mL) giving 3.83 g of product, mp 259-261 °C (dec.). Recrystallization from 0.1 M perchloric acid gave 3.43 g (8.26 mmoL, 83%) of white crystals, mp 261.5-263.5 °C (dec.). An analytical sample was prepared by three recrystallizations from 0.1 M perchloric acid followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h, mp 262-263.5 °C (dec.): ^{1}H NMR (c. 0.36 M, Me_2SO-d_6, Me_4Si) δ2.63 (2'-CH_3), 3.82 (NCH_3), 5.75 (CH_2), 8.19 (H_3, H_5, dd, $J_{2,3} = J_{5,6} = 7$ Hz, $J_{3,4} = J_{4,5} = 9$ Hz), 8.49 (H_6'), 8.68 (H_4, t, J = 9 Hz), 9.03 (H_2, H_6, d, J = 7 Hz), 8.80, 9.28 (NH_2); ^{13}C NMR (c. 0.36 M, Me_2SO-d_6, Me_4Si) δ21.6 (2'-CH_3), 42.0 (NCH_3), 56.0 (CH_2), 106.8 (C_5'), 128.1 (C_3, C_5), 144.5, 146.4 (C_2, C_6, C_4), 151.4 (C_6'), 161.8, 163.1 (C_2', C_4').

Anal. Calcd for $C_{12}H_{16}Cl_2N_4O_8$ (415.2): C, 34.71; H, 3.88; N, 13.49. Found: C, 34.67; H, 3.90; N, 13.49.

Preparation of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)-methyl]-2-aminopyridinium Diperchlorate (33). To a solution of 1'-methylthiaminium dichloride (1.00 g, 2.85 mmoL) in methanol (20 mL) was added 2-aminopyridine (0.355 g, 3.77 mmoL) in two portions. After addition of the first portion (0.273 g, 2.90 mmoL) the solution was stirred at ambient temperature for 12 h whereupon the second portion (0.082 g, 0.87 mmoL) was added and stirring continued for an additional 12 h. Addition of sodium perchlorate (2.50 g, 20.4 mmoL) precipitated the organic cations as perchlorate salts along with sodium chloride. The mixture of solids was recrystallized four times from 0.1 M perchloric acid to give 0.470 g

(1.09 mmoL, 38%) of a white crystalline product, mp 230-234 °C (dec.). A sample for analysis was twice recrystallized from 0.1 M perchloric acid followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h, mp 236-238 °C (dec.): ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.61 (2'-CH_3), 3.79 (NCH_3), 5.00 (CH_2), 6.90 (H_5, dd, $J_{4,5} \cong J_{5,6} \cong 7$ Hz), 7.16 (H_3, d, $J_{3,4} = 9$ Hz), 7.90 (H_4, H_6, mult.), 8.00 (H_6'), 8.57 (2-NH_2), 8.68, 9.20 (4'-NH_2), (DCl, pD<1, DSS) δ2.75 (2'-CH_3), 3.90 (NCH_3), 5.35 (CH_2), 7.08 (H_5, dd, $J_{4,5} \cong J_{5,6} \cong 7$ Hz), 7.33 (H_3, d, $J_{3,4} = 9$ Hz), 7.78 (H_6'), 7.87 (H_6, d, $J_{5,6} = 7$ Hz), 8.04 (H_4, dd, $J_{3,4} = 9$ Hz, $J_{4,5} = 7$ Hz); (D_2O, pD 7.08, DSS) δ2.72 (2'-CH_3), 3.85 (NCH_3), 5.36 (CH_2), 7.06 (H_5, dd, $J_{4,5} \cong J_{5,6} \cong 7$ Hz), 7.33 (H_3, d, $J_{3,4} = 9$ Hz), 7.65 (H_6'), 7.83 (H_6, d, $J_{5,6} = 7$ Hz), 8.03 (H_4, dd, $J_{3,4} = 9$ Hz, $J_{4,5} = 7$ Hz); (c. 0.15 M, H_2O, pH 9.23, DSS) δ2.71 (2'-CH_3), 3.85 (NCH_3), 5.30 (CH_2), 7.03 (H_5, dd, $J_{4,5} \cong J_{5,6} = 6$ Hz), 7.23 (H_3, d, $J_{3,4} = 9$ Hz), 7.67 (H_6'), 7.80 (H_6, d, $J_{5,6} = 6$ Hz), 7.93 (H_4, dd, $J_{3,4} = 9$ Hz, $J_{4,5} = 6$ Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ21.8 (2'-CH_3), 42.2 (NCH_3), 49.7 (CH_2), 107.4 (C_5'), 113.7, 116.0 (C_3, C_5), 137.8, 143.0 (C_4, C_6), 148.1 (C_6'), 157.1 (C_2), 162.5, 163.5 (C_2', C_4').

Anal. Calcd for $C_{12}H_{17}Cl_2N_5O_8$ (430.2): C, 33.50; H, 3.98; N, 16.28. Found: C, 33.43; H, 4.02; N, 16.26.

Preparation of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)-methyl]-3-aminopyridinium Diperchlorate (54). 1'-Methylthiaminium diperchlorate (2.50 g, 5.22 mmoL) suspended in methanol (25 mL) was heated at reflux with 3-aminopyridine

(1.00 g, 10.6 mmoL) for 24 h during which time the suspension dissolved. The solvent was removed under reduced pressure and the residue was triturated with ethyl acetate (6 x 20 mL) to give 2.31 g of a brown material, mp 120-180 °C. Recrystallization from 0.1 M perchloric acid and charcoal gave 1.19 g (2.77 mmoL, 53%) of a white crystalline product, mp 203-205 °C. An analytical sample was prepared by two further recrystallizations from 0.1 M perchloric acid followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h, mp 207-209 °C: ^{1}H NMR (c. 0.19 M, Me_2SO-d_6, Me_4Si) δ2.63 (2'-CH_3), 3.81 (NCH_3), 5.57 (CH_2), 6.95 (3-NH_2), 7.68 (H_4, H_5, mult.), 8.07 (H_2, H_6, mult.), 8.47 (H_6'), 8.82, 9.32 (4'-NH_2); ^{13}C NMR (c. 0.35 M, Me_2SO-d_6, Me_4Si) δ21.6 (2'-CH_3), 41.9 (NCH_3), 55.5 (CH_2), 107.4 (C_5'), 127.9, 128.0, 130.6 (C_2, C_4, C_5, C_6), 148.5 (C_3), 150.8 (C_6'), 161.9, 163.0 (C_2', C_4').

Anal. Calcd for $C_{12}H_{17}Cl_2N_5O_8$ (430.2): C, 33.50; H, 3.98; N, 16.28. Found: C, 33.61; H, 3.99; H, 16.31.

Preparation of 1-[(4-Amino-1,2-dimethyl-5-pyrimidino)-methyl]-4-aminopyridinium Diperchlorate (12). A suspension of 1'-methylthiaminium diperchlorate (2.50 g, 5.22 mmoL) and 4-aminopyridine (0.540 g, 5.74 mmoL) in methanol (30 mL) was stirred at ambient temperature for 16 h. The mixture was filtered and the product was washed with ethyl acetate (2 x 5 mL) giving 1.92 g of a white solid, mp 198-201 °C. Recrystallization from 0.1 M perchloric acid gave 1.58 g (3.67 mmoL, 70%) of white crystals, mp 198-201 °C. An analytical sample was prepared by recrystallization,

twice from 0.1 M perchloric acid followed by recrystallization from water and twice from 0.1 M perchloric acid with vacuum drying at 100 °C over magnesium perchlorate for 12 h. On heating there was slight melting at 198-200 °C with some resolidification above 205 °C and final melting at 215.5-217.5 °C.

Anal. Calcd for $C_{12}H_{17}Cl_2N_5O_8$ (430.2): C, 33.50; H, 3.98; N, 16.28. Found: C, 33.47; H, 4.02; N, 16.28.

Over a period of months the melting point of the analytical sample changes such that the slight melting at 198-200 °C becomes predominant though not complete; a small amount of material remains solid to 213 °C. Upon drying under vacuum at 100 °C over magnesium perchlorate for 48 h the melting at 198-200 °C again becomes only slight although not negligible. A sample recrystallized from water and air dried melts completely between 196 and 199 °C. The presence of small amounts of water either results in hydrate formation or alters the crystal form of the product. Proton and carbon NMR samples prepared from material with a melting point of 198-200 °C are consistent with the proposed structure. ^{1}H NMR (c. 0.35 M, Me_2SO-d_6, Me_4Si) δ2.60 (2'-CH_3), 3.80 (NCH_3), 5.23 (CH_2), 6.85 (H_3, H_5, apparent AB, $J_{2,3} \cong 7$ Hz), 8.15 (H_2, H_6, apparent AB, $J_{2,3} \cong 7$ Hz), 8.18 (4-NH_2), 8.31 (H_6'), 8.67, 9.08 (4'-NH_2); ^{13}C NMR (c. 0.35 M, Me_2SO-d_6, Me_4Si) δ21.5 (2'-CH_3), 41.9 (NCH_3), 52.3 (CH_2), 109.0, 109.5 (C_5', C_3, C_5), 142.6 (C_2, C_6), 149.5 (C_6'), 158.9 (C_4), 161.6, 162.7 (C_2', C_4').

Preparation of 4-Amino-1,2-dimethyl-5-[1,4-triethylenediammonio]methylpyrimidinium Triperchlorate (55). A suspension of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) and 1,4-triethylenediamine hydrate (3.51 g, 27.0 mmoL) in methanol (50 mL) was stirred at ambient temperature for 4 h. The resulting mixture was cooled in ice for 30 min and filtered, followed by washing first with methanol (2 x 10 mL) then ethyl acetate (2 x 10 mL) and finally ethyl ether (2 x 10 mL). The hygroscopic solid was dissolved in hot 3.9 M perchloric acid (15 mL). The recrystallized protonated product was filtered, washed with ice-cold water (2 x 10 mL) and suspended in absolute ethanol (30 mL) for 30 min. The product was collected and air dried to give 4.60 g (8.38 mmoL, 81%) of white crystals, mp 264.5-266.5 °C (dec.) with prior elimination of water at 150 °C. A sample for analysis was prepared by recrystallization from 0.1 M perchloric acid, then from 1.2 M perchloric acid followed by vacuum drying at 100 °C over anhydrous magnesium perchlorate for 3 h and ambient temperature for 15 h, mp 268-270 °C (dec.) with elimination of water at 150 °C. A small portion of the analytical sample was not vacuum dried but air dried for 18 h, mp 265-268 °C (dec.): ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.63 (2-CH_3), 3.70 (2'-CH_2, 3'-CH_2, broad), 3.83 (NCH_3), 4.67 (5-CH_2), 8.47 (H_6), 8.93, 9.40 (NH_2); ^{13}C NMR (c. 0.35 M, Me_2SO-d_6, Me_4Si) δ21.6 (2-CH_3), 42.0 (NCH_3), 43.2, 49.8, 59.4 (2'-CH_2, 3'-CH_2, 5-CH_2), 101.9 (C_5), 153.8 (C_6), 163.1, 163.2 (C_2, C_4). Anal. Calcd for $C_{13}H_{24}N_5Cl_3O_{12}\cdot H_2O$ (566.7): C, 27.55; H, 4.62; N, 12.36. Found: C, 27.62; H, 4.66; N, 12.40.

Preparation of 4-Amino-1,2-dimethyl-5-[1-piperidino]-methylpyrimidinium Perchlorate (56). A suspension of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) and piperidine (2.66 g, 31.2 mmoL) in methanol (50 mL) was stirred at ambient temperature for 90 min. The solvent was removed under reduced pressure leaving a slightly oily residue. Trituration of the residue with ethyl acetate (5 mL) followed by filtration gave a slightly yellow solid. Washing with ethyl acetate (5 x 3 mL) produced 2.02 g of an off-white solid, mp 157-162 °C. Recrystallization from a 4:1 (v/v) mixture of ethyl acetate and methanol with charcoal gave 1.69 g (5.27 mmoL, 51%) of colorless hexagonal platelets, mp 161-163 °C. A sample for analysis was prepared by recrystallization from ethyl acetate/methanol followed by vacuum drying at ambient temperature over magnesium perchlorate for 16 h, mp 161-162.5 °C: ^{1}H NMR (c. 0.69 M, Me_2SO-d_6, Me_4Si) δ1.45 (3', 5'-CH_2, 4'-CH_2), 2.36 (2', 6'-CH_2), 2.55 (2-CH_3), 3.36 (5-CH_2), 3.78 (NCH_3), 8.15 (H_6), 8.2-9.2 (NH_2, broad); ^{13}C NMR (c. 0.69 M, Me_2SO-d_6, Me_4Si), δ21.4 (2-CH_3), 23.7, 24.5 (C_3', C_5', C_4'), 41.6 (NCH_3), 53.4 (C_2', C_6'), 55.6 (5-CH_2), 112.3 (C_5), 146.4 (C_6), 161.5, 162.9 (C_2, C_4).

Anal. Calcd for $C_{12}H_{21}ClN_4O_4$ (320.8): C, 44.93; H, 6.60; N, 17.47. Found: C, 45.03; H, 6.65; N, 17.49.

Preparation of 4-Amino-1,2-dimethyl-5-[(6-methyl-2-pyridinyl)amino]methylpyrimidinium Perchlorate (35) and Its Conjugate Acid (35'). To a suspension of 1'-methylthiaminium diperchlorate (2.22 g, 4.63 mmoL) in methanol (30 mL) was

added 2-amino-6-methylpyridine (1.25 g, 11.6 mmoL) and the mixture was heated at reflux for 24 h. After cooling in ice for 30 min the mixture was filtered and the product washed with ice-cold methanol (2 x 5 mL) to give 1.43 g (4.16 mmoL, 90%) of white crystals, mp 264-266 °C (dec.). Recrystallization of 0.43 g of product from 20% (v/v) aqueous ethanol gave 0.35 g of white crystals, mp 261-264 °C (dec.). Recrystallization of 1.00 g of product from acetonitrile yielded 0.88 g of crystals, mp 264-266 °C (dec.). A sample for analysis was recrystallized three times from water and vacuum dried at 100 °C for 2 h over magnesium perchlorate, mp 262-263 °C (dec.): ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.31 (6'-CH_3), 2.57 (2-CH_3), 3.81 (NCH_3), 4.33 (CH_2, d, $J_{CH_2,NH}$ = 6 Hz), 6.39 (H_5', d, J_4', 5', = 8 Hz), 6.45 (H_3', d, H_3',$_5$' = 8 Hz), 6.88 (2'-NH, t, $J_{CH_2,NH}$ = 6 Hz), 7.34 (H_4', dd, J_4',$_5$' = 8 Hz, J_3',$_5$' = 7 Hz), 8.21 (H_6), 9.06 (4-NH_2); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ21.4 (2-CH_3), 23.7 (6'-CH_3), 38.1 (CH_2), 41.6 (NCH_3), 105.9, 111.7 (C_3', C_5'), 114.5 (C_5), 137.8 (C_4'), 146.6 (C_6), 155.3, 157.6 (C_2', C_6'), 161.5, 162.5 (C_2, C_4). Anal. Calcd for $C_{13}H_{18}ClN_5O_4$ (343.8): C, 45.42; H, 5.28; N, 20.37. Found: C, 45.60; H, 5.34; N, 20.42.

The conjugate acid was produced upon recrystallization of 1.44 g (4.18 mmoL) from 20 mL of 0.1 M perchloric acid and 15 drops of 11.8 M perchloric acid to give 1.24 g (3.03 mmoL, 72%) of white crystals, mp 203-205 °C (dec.). Recrystallization from 0.1 M perchloric acid followed by vacuum drying at 100 °C for 5 h over magnesium perchlorate

gave the analytical sample, mp 202-204 °C (dec.): ^{1}H NMR (c. 0.45 M, Me_2SO-d_6, Me_4Si) δ2.53 (2-CH_3), 2.62 (6'-CH_3), 3.82 (NCH_3), 4.43 (CH_2, broad), 6.80 (H_3', d, J_3', $_4$' = 7 Hz), 6.93 (H_5', d, J_4',$_5$' = 9 Hz), 7.91 (H_4', dd, J_4',$_5$' = 9 Hz, J_3',$_5$' = 7 Hz), 8.27 (H_6), 8.53, 9.18 (4-NH_2), 8.17 (2'-NH, broad), 12.17 (NH, broad); ^{13}C NMR (c. 0.45 M, Me_2SO-d_6, Me_4Si) δ19.0 (6'-CH_3), 21.4 (2-CH_3), 39.2 (CH_2), 41.7 (NCH_3), 111.2 (C_5), 109.2, 112.8 (C_3', C_5'), 144.2, 145.8 (C_4', C_6), 147.8, 153.0 (C_2', C_6'), 161.8, 162.0 (C_2, C_4).

Anal. Calcd for $C_{13}H_{19}Cl_2N_5O_8$ (444.2): C, 33.15; H, 4.31; H, 15.77. Found: C, 35.12; H, 4.33; N, 15.75.

Preparation of 4-Amino-1,2-dimethyl-5-azidomethylpyrimidinium Perchlorate (57). Sodium azide (0.500 g, 7.69 mmoL) and 1'-methylthiaminium diperchlorate (2.50 g, 5.22 mmoL) were stirred together in methanol (30 mL) for 16 h at ambient temperature. NMR analysis showed a small amount of unreacted starting material which was then converted to product by brief warming (10 min) at 50 °C. Anhydrous sodium perchlorate (1.22 g, 10.0 mmoL) was added and the volume of solution was concentrated to 15 mL with a slow stream of nitrogen. After a few hours rectangular plates of product crystallized. The product was collected by filtration and washed with ice-cold methanol to give 0.570 g of colorless crystals, mp 109-111 °C. Further concentration of the mother liquor to 5 mL produced 0.47 g of product, mp 109-110 °C. Recrystallization of the combined yield of product from methanol and sodium perchlorate (0.150 g, 1.23 mmoL) gave 0.550 g (1.97 mmoL, 38%) of product, mp

111-114 °C. A sample for analysis was recrystallized from methanol and vacuum dried at ambient temperature over magnesium perchlorate for 8 h, mp 114.5-116.5 °C: ^{1}H NMR (D_2O, DSS) δ2.63 (2-CH_3), 3.88 (NCH_3), 4.48 (CH_2), 8.15 (H_6); ^{13}C NMR (D_2O, DSS) δ24.0 (2-CH_3), 44.8 (NCH_3), 49.6 (CH_2), 114.0 (C_5), 150.0 (C_6), 164.9, 166.0 (C_2, C_4). Anal. Calcd for $C_7H_{11}ClN_6O_4$ (287.7): C, 30.17; H, 3.98; N, 30.06. Found: C, 30.15; H, 4.02; N, 30.06.

Preparation of 4-Amino-1,2-dimethyl-5-thiophenoxymethylpyrimidinium Perchlorate (58). Thiophenol (1.15 g, 1.06 mL, 20.8 mmoL) was added by syringe to a suspension of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) in methanol (50 mL). Methanolic sodium hydroxide (0.41 g, 10.3 mmoL, 5.0 mL) was added and the mixture heated at reflux for 2 h. After cooling in ice for 1 h the mixture was filtered and washed first with ice-cold methanol (2 x 10 mL) and then with ethyl ether (2 x 10 mL) giving 2.52 g of a white crystalline product, mp 155-158.5 °C. Recrystallization from 10% aqueous dimethylformamide yielded 2.02 g (5.84 mmoL, 56%) of product, mp 155.5-157.5 °C. An analytical sample was prepared by further recrystallization from 10% aqueous dimethylformamide followed by vacuum drying at ambient temperature over phosphorus pentoxide for 12 h, mp 157-159 °C: ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.55 (2-CH_3), 3.73 (NCH_3), 4.25 (CH_2), 7.19-7.39 (aromatic mult.), 8.28 (H_6), 9.04, 9.28 (NH_2); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ21.5 (2-CH_3), 29.7 (CH_2), 41.4 (NCH_3), 111.9 (C_5), 126.7 (C_4'), 129.1 (C_2', C_6'), 130.1 (C_3', C_5'), 134.0 (C_1'), 146.4 (C_6), 161.3, 161.5 (C_2, C_4).

Anal. Calcd for $C_{13}H_{16}ClN_3O_4S$ (345.8): C, 45.15; H, 4.66; N, 12.15. Found: C, 45.22; H, 4.65; N, 12.17.

Preparation of 4-Amino-1,2-dimethyl-5-[2-pyridinylthio]-methylpyrimidinium Perchlorate (59). A suspension of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) and 2-thiopyridone (2.50 g, 22.5 mmoL) in methanol (50 mL) was heated at reflux. Methanolic sodium hydroxide (0.516 g, 12.9 mmoL in 3.00 mL) was added and heating continued for 20 min. The solvent was removed under reduced pressure and the residue triturated with ethyl acetate (3 x 10 mL). The resulting solid was collected and washed first with ethyl acetate (10 mL) then with ethyl ether (2 x 10 mL) to give 3.15 g of product, mp 192-197 °C. Recrystallization from water gave 2.76 g (7.96 mmoL, 77%) of slightly yellow needles, mp 194-197 °C. A sample for analysis was prepared by further recrystallization from water followed by vacuum drying at 100 °C over magnesium perchlorate for 4 h, mp 195.5-196.5 °C: ^{1}H NMR (c. 0.58 M, Me_2SO-d_6, Me_4Si) δ2.57 (2-CH_3), 3.80 (NCH_3), 4.33 (CH_2), 7.20 (H_5', dd, $J_{4',5'}$ = 8 Hz, $J_{5',6'}$ = 5 Hz), 7.37 (H_3', d, $J_{3',4'}$ = 7 Hz), 7.58 (H_4', dd, $J_{3',4'}$ = 7 Hz, $J_{4',5'}$ = 8 Hz), 8.40 (H_6), 8.55 (H_6', d, $J_{5',6'}$ = 5 Hz), 8.67, 9.08 (NH_2); ^{13}C NMR (c. 0.58 M, Me_2SO-d_6, Me_4Si) 21.3 (2-CH_3), 25.9 (CH_2), 41.7 (NCH_3), 112.8 (C_5), 120.8, 122.8 (C_3', C_5'), 137.7, 148.1, 150.1 (C_4' C_6', C_6), 157.4 (C_2'), 162.4, 162.7 (C_2, C_4). Anal. Calcd for $C_{12}H_{15}ClN_4O_4S$ (346.8): C, 41.56; H, 4.36; N, 16.16. Found: C, 41.59; H, 4.39; N, 16.15.

Preparation of 4-Amino-1,2-dimethylpyrimidinium-5-methylsulfonate (60). 1'-Methylthiaminium diperchlorate (2.11 g, 6.01 mmoL) and sodium sulfite (0.760 g, 6.03 mmoL) in water (10 mL) were stirred at ambient temperature for 15 min. The precipitate which had begun to form on mixing was filtered and washed first with ice-cold water (5 mL) and then with acetone (5 mL) to give 1.10 g of a white powder, mp > 310 °C. Recrystallization from water yielded 0.91 g (4.19 mmoL, 70%) of product, mp > 310 °C. A sample for analysis was further recrystallized from water followed by vacuum drying at 100 °C over magnesium perchlorate, mp > 310 °C: ^{1}H NMR (c. 0.46 M, D_2O, 1.6 M $NaClO_4$[a], TSP) δ2.67 (2-CH_3), 3.91 (NCH_3), 4.04 (CH_2), 8.18 (H_6); ^{13}C NMR (c. 0.46 M, D_2O, 1.6 M $NaClO_4$, 80 °C, TSP) δ44.8 (NCH_3), 51.9 (CH_2), 111.6 (C_5), 152.1 (C_6), 163.4 (C_2, C_4), 2-CH_3 underwent H/D exchange.

Anal. Calcd for $C_7H_{11}N_3O_3S$ (217.2): C, 38.70; H, 5.10; N, 19.34. Found: C, 38.68; H, 5.10; N, 19.30.

Preparation of 4-Amino-1,2-dimethyl-5-(phenylsulfonylmethyl)pyrimidinium Perchlorate (30). A suspension of 1'-methylthiaminium diperchlorate (1.00 g, 2.09 mmoL), sodium benzenesulfinate (1.00 g, 6.09 mmoL) and 2,4,6-trimethylpyridine (1.00 mL, 1.09 g, 9.00 mmoL) in methanol (5 mL) was heated in a screw-cap vial at 100 °C for 45 min. The

(a) supersaturates in the presence of high salt concentrations.

resulting solution was cooled in ice for 1 h which resulted in precipitation. The crude product was isolated by filtration and washed first with ethanol (3 x 5 mL) then ethylacetate (3 x 5 mL). After air drying the yield of crude product was 0.55 g (78%, 1.63 mmoL), mp 205-207.5 °C. Recrystallization from an 8:1 (v/v) mixture of ethanol and 0.1 M perchloric acid gave 0.22 g (31%, 0.65 mmoL) of product, mp 209-211 °C. The large reduction in yield upon recrystallization was primarily due to loss in handling. A sample for analysis was prepared by recrystallization first from a 9.5:1 (v/v) mixture of 0.1 M perchloric acid and ethanol, then from 90% ethanol followed by vacuum drying at 100 °C over magnesium perchlorate for 3 h, mp 209-210.5 °C: ^{1}H NMR (c. 0.16 M, Me_2SO-d_6, Me_4Si) δ2.53 (2-CH_3), 3.78 (NCH_3), 4.68 (CH_2), 7.87 (aromatic multiplet), 8.20 (H_6), 8.40, 9.20 (NH_2).

Anal. Calcd for $C_{13}H_{16}N_3ClSO_6$ (377.8): C, 41.33; H, 4.27; N, 11.12. Found: C, 41.35; H, 4.31; N, 11.13.

Preparation of 4-Amino-1,2-dimethyl-5-[triphenylphosphoniomethyl]pyrimidinium Diperchlorate (29). Triphenylphosphine (4.91 g, 18.7 mmoL), 1'-methylthiaminium diperchlorate (3.00 g, 6.24 mmoL), and 2,4,6-trimethylpyridine (3.00 mL, 3.27 g, 27.0 mmoL) in methanol (50 mL) were heated at reflux for 4 h. The resulting mixture was cooled in ice for 30 min. The crude product was collected by filtration and washed with ethylacetate (3 x 10 mL) to give 3.08 g of a white solid, mp 298-300 °C (dec.). Recrystallization from water gave 2.85 g (76%, 4.76 mmoL) of white needles, mp 300-

303 °C (dec.). An analytical sample was recrystallized from water and vacuum dried at 100 °C over magnesium perchlorate for 3 h; mp 303-305 °C (dec.): ^{1}H NMR ([3:2 (v/v) Me_2SO-d_6/D_2O, Me_4Si] δ2.43 (2-CH_3), 3.67 (NCH_3), 4.91 (CH_2, d, J^2_{PH} = 14 Hz), 7.90 (H_6, aromatic multiplet); ^{13}C NMR ($MeNO_2$-d_3, Me_4Si) δ22.5 (2-CH_3), 23.8 (CH_2, d, J_{CP} = 52 Hz), 43.6 (NCH_3), 105.3 (C_5, d, J^2_{CP} = 7 Hz), 117.3 (C_1', C_5', d, J^3_{CP} = 10 Hz), 137.2 (C_4'), 151.7 (C_6), 164.8, 164.8 (C_2, C_4). Anal. Calcd for $C_{25}H_{26}Cl_2N_3O_8P$ (598.4): C, 50.18; H, 4.38; N, 7.02. Found: C, 50.01; H, 4.42; N, 6.97.

Heating the proton NMR sample at 100 °C for 10 min resulted in complete H/D exchange of the methylene group.

Preparation of 2,3-Dimethyl-5H-pyrido[1,2-a]pyrimido-[4,5-d]pyrimidinium Perchlorate (34) and Its Conjugate Acid (34'):[2,3-Dimethylpyrichrominium Perchlorate, (34')]. A suspension of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) and 2-aminopyridine (2.54 g, 26.0 mmoL) in methanol (50 mL) was heated at reflux for 24 h. The suspension was cooled in ice for 30 min, filtered, and the crude product washed with ice-cold methanol (2 x 5 mL) to give 2.78 g of yellow needles, mp 241-244 °C (dec.). Recrystallization from water yielded 2.54 g (8.12 mmoL, 78%) of yellow needles, mp 248-249 °C (dec.). A sample for analysis was recrystallized three times from water followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h, mp 245-246.5 °C (dec.): UV (c. 3.36 x 10^{-5} M, H_2O, pH 6.86) λmax (log ε) 397 (4.33), 288 (4.02), 251 (3.73), 220 (sh), 202 (4.45); Fluorescence (c. 3.36 x 10^{-7} M, H_2O, pH 6.86) λemission (λexcitation)

431, 448_{sh} (400): ^{1}H NMR (c. 0.10 M, Me_2SO-d_6, Me_4Si) δ2.57 (2-CH_3), 3.78 (NCH_3), 5.58 (CH_2, d, $J_{CH_2,H_4} \cong 1.5$ Hz), 7.19 (H_{10}, d, $J_{9,10} = 6$ Hz), 7.16 (H_8, dd, $J_{7,8} \cong J_{8,9} \cong 6$ Hz), 7.94 (H_9, dd, $J_{8,9} \cong J_{9,10} \cong 6$ Hz), 8.08 (H_7, s, $J_{7,8} = 6$ Hz), 8.10 (H_4, t, $J_{CH_2,H_4} \cong 1.5$ Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ21.7 (2-CH_3), 41.8 (NCH_3), 49.7 (CH_2), 108.8 (C_{4a}), 117.0 (C_8), 123.5 (C_{10}), 140.3 (C_7), 142.5 (C_4), 143.2 (C_9), 155.5 (C_{10a}), 162.0, 162.8 (C_2, C_{11a}); ^{15}N NMR (c. 0.96 M, 7/1 (v/v) CF_3CH_2OH/CH_3NO_2, CrAcAc, shifts relative to CH_3NO_2) δ122.1 (N_{11}), 186.7 (N_1), 216.2 (N_3), 217.4 (N_6).

Anal. Calcd for $C_{12}H_{13}ClN_4O_4$ (312.7): C, 46.09; H, 4.19; N, 17.92. Found: C, 45.87; H, 4.26; H, 17.82.

The product was synthesized in 66% yield by sealing the starting materials in a thick-wall glass ampoule in methanol (20 mL) and heating at 100 °C for 2 h, mp 246.5-248 °C after recrystallization.

Melting points were dependent on the heating rate used for the determination. Slow heating from ambient temperature resulted in a melting point of 241-243 °C (dec.) while heating the sample from an initial temperature of 215 °C gave a melting point of 246.5-248 °C (dec.).

The conjugate acid was prepared by recrystallization of 34 (0.500 g, 1.60 mmoL) from 0.1 M perchloric acid (20 mL) to which had been added 11.8 M perchloric acid (10 drops). The yield was 0.541 g (1.31 mmoL, 82%) of white crystals, mp 298-300.5 °C (dec.): UV (c. 4.56×10^{-5} M, H_2O, pH 1.00) λmax (log ε) 343 (4.23), 266 (4.02), 244 (3.87), 217 (sh)

200 (4.48): ^{1}H NMR (Me_2SO-d_6. Me_4Si) δ2.87 (2-CH_3), 4.07 (NCH_3), 5.87 (CH_2), 7.57 (H_{10}, d, $J_{9,10}$ = 6 Hz), 7.67 (H_8, dd, $J_{7,8} \cong 8$ Hz, $J_{8,9} \cong 7$ Hz), 8.38 (H_7, d, $J_{7,8}$ = 8 Hz), 8.54 (H_9, dd, $J_{8,9}$ = 7 Hz, $J_{9,10}$ = 6 Hz), 8.89 (H_4).

Preparation of 2,3-Dimethyl-5H-pyrido[1,2-a]pyrimido-[4,5-d]pyrimidinium Perchlorate (34) via 2-Methoxypyridine. A suspension of 1'-methylthiaminium diperchlorate (1.00 g, 2.09 mmoL), 2-methoxypyridine (1.14 g, 10.4 mmoL) and 2,4,6-trimethylpyridine (0.560 g, 4.62 mmoL) in methanol (25 mL) was heated at reflux for 24 h. The resulting mixture was ice-cooled for 45 min, filtered and the crude product washed with ice-cold methanol to give 0.496 g of yellow crystals, mp 238-239 °C (dec.). Recrystallization from water gave 0.480 g of yellow needles, mp 245-247 °C (dec.). The mixed melting point of the product before purification with authentic 34 was 236-238 °C (dec.). The proton NMR was identical to authentic 34.

Preparation of 2,3,10-Trimethyl-5H pyrido[1,2-a]pyrimido-[4,5-d]pyrimidinium Perchlorate (61) and Its Conjugate Acid (61'):[2,3,10-Trimethylpyrichrominium Perchlorate, (61)]. A suspension of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) and 2-amino-3-methylpyridine (2.82 g, 26.0 mmoL) in methanol (50 mL) was heated at reflux for 24 h. After cooling in ice for 30 min the crude product was isolated by filtration and washed with ice-cold methanol (2 x 5 mL) to give 3.02 g of yellow crystals, mp 260-265 °C (dec.). Recrystallization from water yielded 2.48 g (7.59 mmoL, 73%) of yellow cube-like crystals, mp 268.5-270 °C (dec.). A

sample for analysis was prepared by recrystallization from water followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h, mp 268.5-270 °C (dec.): UV (c. 2.45 x 10^{-5} M, H_2O, pH 6.86) λmax (log ε) 400 (4.35), 287 (4.01), 253 (3.91), 223 (4.12), 203 (4.40); Fluorescence (c. 2.45 x 10^{-7} M, H_2O, pH 6.86) λemission (λexcitation) 437, $458_{(sh)}$ (402): ^1H NMR (Me_2SO-d_6, Me_4Si) δ2.26 ($10\text{-}CH_3$), 2.58 ($2\text{-}CH_3$), 3.79 (NCH_3), 5.57 (CH_2, d, $J_{CH_2,H_4} \cong 1.5$ Hz), 7.10 (H_8, dd, $J_{8,9} = 8$ Hz, $J_{7,8} = 6$ Hz), 7.87 (H_9, d, $J_{8,9} = 8$ Hz), 7.94 (H_7, d, $J_{7,8} = 6$ Hz), 8.13 (H_4, t, $J_{CH_2,H_4} \cong 1.5$ Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ17.5 ($10\text{-}CH_3$), 21.7 ($2\text{-}CH_3$), 41.7 (NCH_3), 49.8 (CH_2), 108.9 (C_{4a}), 116.3 (C_8), 131.8 (C_{10}), 138.1 (C_7), 142.0 (C_4), 142.5 (C_9), 154.2 (C_{10a}), 162.0, 162.2 (C_2, C_{11a}).

Anal. Calcd for $C_{13}H_{15}ClN_4O_4$ (326.7): C, 47.79; H, 4.63; N, 17.15. Found: C, 47.75; H, 4.67; N, 17.13.

The conjugate acid was prepared by recrystallization of 61 (0.500 g, 1.53 mmoL) from 0.1 M perchloric acid (20 mL) to which was added 11.8 M perchloric acid (15 drops). The yield was 0.328 g (0.768 mmoL, 50%) of white crystals, mp 284-285 °C (dec.): UV (c. 5.00 x 10^{-5} M, H_2O, pH 1.00) λmax (log ε) 345 (4.21), 264 (3.99), 247 (3.82), $220_{(sh)}$, 202 (4.47); Fluorescence (c. 5.00 x 10^{-7} M, H_2O, pH 1.00) λemission (λexcitation) 437, 460 (sh) (350); ^1H NMR (c. 0.46 M, Me_2SO-d_6, Me_4Si) δ2.50 ($10\text{-}CH_3$), 2.85 ($2\text{-}CH_3$), 4.09 (NCH_3), 5.90 (CH_2), 7.97 (H_8, dd, $J_{7,8} \cong J_{8,9} \cong 7$ Hz), 8.33 (H_9, d, $J_{8,9} = 7$ Hz), 8.51 (H_7, d, $J_{7,8} = 7$ Hz), 8.93 (H_4), 10.80 ($N_{11}H$).

Preparation of 2,3,9-Trimethyl-5H-pyrido[1,2-a]pyrimido-[4,5-d]pyrimidinium Perchlorate (37) and Its Conjugate Acid (37'):[2,3,9-Trimethylpyrichrominium Perchlorate, (37)]. 1'-Methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) was suspended in methanol (50 mL) and heated at reflux with 2-amino-4-methylpyridine (2.82 g, 26.0 mmoL) for 24 h. The resulting mixture was cooled in ice for 30 min, filtered, and the crude product washed with ice-cold methanol (2 x 5 mL) to give 2.58 g of yellow crystals, mp 238-243 °C (dec.). Recrystallization from water yielded 2.03 g (6.21 mmoL, 61%) of yellow needles, mp 245-247 °C: UV (c. 2.48 x 10^{-5} M, H_2O, pH 6.86) λmax (log ε), 391 (4.36), 288 (3.92), 230 (4.24), 204 (4.47; Fluorescence (c. 2.48 x 10^{-7} M, H_2O, pH 6.86) λemission (λexcitation) 425, 442 (sh) (398); ^1H NMR (Me_2SO-d_6, Me_3Si) δ2.37 (9-CH_3), 2.55 (2-CH_3), 3.77 (NCH_3), 5.51 (CH_2, d, $J_{CH_2,H_4} \cong$ 1.5 Hz), 7.00 (H_{10}), 7.04 (H_8, d, $J_{7,8}$ = 7 Hz), 7.96 (H_7, d, $J_{7,8}$ = 7 Hz), 8.07 (H_4, t, $J_{CH_2,H_4} \cong$ 1.5 Hz); ^{13}C NMR (Me_2So-d_6, Me_4Si) δ20.8 (9-CH_3), 21.8 (2-CH_3), 41.7 (NCH_3), 49.0 (CH_2), 108.7 (C_8), 118.7 (C_{4a}), 123.3 (C_{10}), 139.5 (C_7), 142.1 (C_4), 154.6 (C_9), 155.7 (C_{10a}), 161.8, 162.6 (C_2, C_{11a}).

Anal. Calcd for $C_{13}H_{15}ClN_4O_4$ (326.7): C, 47.79; H, 4.63; N, 17.15. Found: C, 47.76; H, 4.63; N, 17.16.

The conjugate acid was prepared by recrystallization of 37 (0.50 g, 1.53 mmoL) from 0.1 M perchloric acid (20 mL) and 11.8 M perchloric acid (10 drops). The yield was 0.626 g (1.47 mmoL, 96%) of white crystals, mp 281-293 °C (dec.): UV (c. 4.72 x 10^{-5} M, H_2O, pH 1.00) λmax (log ε)

340 (4.25), 267 (3.89), 230 (4.24), 204 (4.46); ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.55 (9-CH_3), 2.81 (2-CH_3), 4.04 (NCH_3), 5.78 (CH_2), 6.30 (NH, broad), 7.32 (H_{10}), 7.54 (H_8, $J_{7,8}$ = 6 Hz), 8.46 (H_7, $J_{7,8}$ = 6 Hz), 8.88 (H_4); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ21.4, 22.1 (2-CH_3, 9-CH_3), 43.6 (NCH_3), 49.6 (CH_2), 108.8 (C_{4a}), 115.7 (C_8), 122.4 (C_{10}), 141.1, 145.9 (C_7, C_4), 147.8, 155.2 (C_9, C_{10a}), 160.0, 164.4 (C_2, C_{11a}).

Preparation of 8-Bromo-2,3-dimethyl-5H-pyrido[1,2-a]-pyrimido[4,5-d]pyrimidinium Perchlorate (62): (8-Bromo-2,3-dimethylpyrichrominium Perchlorate). A suspension of 1'-methylthiaminium diperchlorate (1.00 g, 2.09 mmoL), 2-amino-5-bromopyridine (1.44 g, 8.35 mmoL), and 2,4,6-trimethyl-pyridine (1.00 mL, 1.09 g, 9.02 mmoL) in methanol (10 mL) was heated at 100 °C in a screw-cap vial for 12 h. Methanol was removed under reduced pressure and the residue triturated with ethyl acetate (3 x 10 mL) to give 0.80 g of a yellow solid, mp 215-220 °C (dec.). Recrystallization from 90% ethanol yielded 0.712 g (87%, 1.82 mmol) of yellow needles, mp 226-228 °C (dec.). A sample for analysis was recrystal-lized twice from 90% ethanol and vacuum dried at 100 °C over magnesium perchlorate for 3 h, mp 227-230 °C: ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.58 (2-CH_3), 3.83 (NCH_3), 5.57 (CH_2), 7.30 (H_{10}, d, $J_{9,10}$ = 10 Hz), 8.13 (H_9, dd, $J_{9,10}$ = 10 Hz, $J_{7,9}$ = 2 Hz), 8.25 (H_4), 8.50 (H_7, d, $J_{7,9}$ = 2 Hz); UV (c. 3.31 x 10^{-5} M, H_2O, pH 6.86) λmax (log ε) 410 (4.34), 302 (4.06), 260 (3.64), 213 (4.38); Fluorescence (c. 3.31 x 10^{-7} M, H_2O, pH 6.86) λemission (λexcitation) 448, $470_{(sh)}$ (410).

Anal. Calcd for $C_{12}H_{12}BrClN_4O_4$ (391.6): C, 36.80; H, 3.09; N, 14.31. Found: C, 36.67; H, 3.13; N, 14.26.

Preparation of 2,3-Dimethyl-5H-pyrimido[4,5-d]thiazolo-[3,2-a]pyrimidinium Perchlorate (36): (2,3-Dimethylthiachrominium Perchlorate). 2-Aminothiazole (2.60 g, 20.6 mmoL) was added to a suspension of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) in methanol (50 mL) and the mixture was heated at reflux for 24 h. After cooling in ice for 30 min the crude product was collected by filtration and washed with ice-cold methanol (2 x 5 mL) to give 3.42 g of yellow crystals, mp 249-254 °C (dec.). Recrystallization from water gave 1.86 g of yellow crystals, mp 256-260 °C (dec.). A second recrystallization yielded 1.50 g (4.71 mmoL, 45%) of product, mp 264-266 °C (dec.). Vacuum drying at 100 °C over magnesium perchlorate for 2 h increased the melting point to 266-268 °C (dec.). An analytical sample was prepared by recrystallizing twice from water and vacuum drying under the above conditions for 4 h, mp 271-273 °C (dec.): UV (c. 2.67 x 10^{-5} M, H_2O, pH 6.86) λmax (log ε) 382 (4.35), 283 (3.34), 230 (3.95), 210 (sh), 202 (4.32); (c. 6.02 x 10^{-5} M, 3M HCl) 337 (4.16), 241 (3.76), 220 (3.94); Fluorescence (c. 2.67 x 10^{-7} M, H_2O, pH 6.86) λemission (λexcitation) 452 (392); ^1H NMR (c. 0.70 M, Me_2SO-d_6, Me_4Si) δ2.61 (2-CH_3), 3.83 (NCH_3), 5.44 (CH_2, d, J_{CH_2,H_4} = 1.7 Hz), 7.24 (H_8, d, $J_{7,8}$ = 5 Hz), 7.46 (H_7, d, $J_{7,8}$ = 5 Hz), 8.21 (H_4, t, J_{CH_2,H_4} = 1.7 Hz); ^{13}C NMR (c. 0.70 M, Me_2SO-d_6, Me_4Si) δ21.8 (2-CH_3), 42.3 (NCH_3), 45.7 (CH_2), 109.2 (C_{4a}), 110.5 (C_7), 130.1 (C_8), 144.8 (C_4), 162.4 (C_2), 162.6 (C_{10a}), 172.6 (C_{9a}).

Anal. Calcd for $C_{10}H_{11}ClN_4O_4S$ (318.7): C, 37.68; H, 3.48; N, 17.58. Found: C, 37.74; H, 3.51; N, 17.54.

Preparation of 2-Amino-6,7-dimethyl-4H-pyrimido[4,5-d]-[1,3]thiazinium Perchlorate (43). A suspension of 1'-methylthiaminium diperchlorate (5.52 g, 11.5 mmoL) and thiourea (4.55 g, 59.8 mmoL) in methanol (30 mL) was sealed in a thick-wall glass bomb and heated at 100 °C for 5 h to produce a crystalline solid. The bomb was cooled in ice for 45 min, opened and the contents filtered. The crude product was washed first with ice-cold methanol (2 x 5 ml) and then with acetone (10 mL) to yield 2.09 g (7.09 mmoL, 62%) of white needles, mp 278-280 °C (dec.), and was used for ^{15}N NMR without further purification. A sample for analysis was recrystallized from 0.1 M perchloric acid and vacuum dried over magnesium perchlorate, mp 290-293 °C (dec.): UV (H_2O) λmax 328, 267, 225; Fluorescence λemission (λexcitation), 395 (355); ^1H NMR (c. 0.20 m, Me_2SO-d_6, Me_4Si) δ2.72 (6-CH_3), 3.95 (NCH_3), 4.33 (CH_2), 8.58 (H_5), 9.63 (NH_2); ^{13}C NMR (c. 1.03 M, Me_2SO-d_6, Me_4Si) 21.9 (6-CH_3), 24.4 (CH_2), 42.6 (NCH_3), 110.1 (C_{4a}), 146.4 (C_5), 162.1, 166.7 (C_7, C_{8a}), 169.0 (C_2); ^{15}N NMR (c. 0.50 M, 20% Me_2SO-d_6 in Me_2SO, CH_3NO_2, c. 1.0 M) δ111.3 (N_1), 157.8 (N_8), 208.1 (N_6), 255.0 (2-NH_2).

Preparation of 3-[(1,2-Dimethyl-3,4-dihydro-3H-4-oxo-5-pyrimidinio)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium Diperchlorate (63). A solution of 1'-methylthiaminium diperchlorate (5.00 g, 10.4 mmoL) in 6 M hydrochloric acid (30 mL) was heated at reflux for 18 h. The solvent was removed under reduced pressure to give a hygroscopic mixture

of product and ammonium salts. Extraction with acetonitrile (6 x 20 mL) followed by evaporation under reduced pressure left the product as a hygroscopic solid. Recrystallization from methanol and sodium perchlorate (5.00 g, 41.0 mmoL) gave 3.01 g (6.27 mmoL, 60%) of white needles, mp 160-163 °C. A second recrystallization from 90% ethanol yielded 2.80 g (5.83 mmoL, 56%) of product, mp 164-166 °C. A sample for analysis was recrystallized twice from methanol and vacuum dried at ambient temperature over magnesium perchlorate for 16 h, mp 164.5-166 °C: ^{1}H NMR (c. 0.50 M, Me_2SO-d_6, Me_4Si) δ2.46, 2.63 (2'-CH_3, 4-CH_3), 2.99 (5-CH_2, t, J = 5 Hz), 3.67 (CH_2OH, t, J = 5 Hz), 3.73 (NCH_3), 5.48 (CH_2), 8.30 (H_6'), 9.95 (H_2), 9.10 (N_3'H, H_2O); ^{13}C NMR (c. 0.50 M, Me_2SO-d_6, Me_4Si) δ11.3 (4-CH_3), 18.7 (2'-CH_3), 29.3 (5-CH_2), 41.4 (NCH_3), 48.9 (5'-CH_2), 158.2, 159.6, 164.7 (C_2, C_2', C_4').

Anal. Calcd for $C_{13}H_{19}Cl_2N_3O_{10}S$ (480.3): C, 32.51; H, 3.99; N, 8.75. Found: C, 32.67; H, 4.05; N, 8.68.

Preparation of 1-[(1,2-Dimethyl-3,4-dihydro-3H-4-oxo-5-pyrimidinio)methyl]pyridinium Diperchlorate (64). A solution of 1-[(4-amino-1,2-dimethyl-5-pyrimidinio)methyl]pyridinium diperchlorate (3.00 g, 7.22 mmoL) in 6 M hydrochloric acid (15 mL) was heated at 100 °C for 18 h. Sodium perchlorate (3.52 g, 28.9 mmoL) was added to the hot solution to insure isolation of the perchlorate salt of the product. Upon cooling, colorless needles formed which proved to be hygroscopic. Recrystallization from methanol gave 1.75 g of colorless needles, mp 248-252 °C (dec.). A second

recrystallization from 90% methanol yielded 1.41 g (3.39 mmoL, 47%) of product, mp 262-265 °C (dec.). A sample for analysis was prepared by recrystallization from 90% methanol followed by vacuum drying at 100 °C over magnesium perchlorate for 5 h, mp 265-266.5 °C (dec.): ^{1}H NMR (c. 0.24 M, Me_2SO-d_6, Me_4Si) δ2.67 (2'-CH_3), 3.77 (NCH_3), 5.66 (CH_2), 8.17 (H_3, H_5, dd, $J_{2,3}$ = 6 Hz, $J_{3,4}$ = 8 Hz), 8.52 (H_6'), 8.65 (H_4, t, $J_{3,4}$ = 8 Hz), 9.09 (H_2, H_6, d, $J_{2,3}$ = 6 Hz), 11.67 (N_3'H).

Anal. Calcd for $C_{12}H_{15}N_3O_9Cl_2$ (416.2): C, 34.63; H, 3.63; N, 10.10. Found: C, 34.68; H, 3.67; N, 10.08.

The melting point was found to be dependent on the rate of heating. Slow heating from ambient temperature resulted in a melting range of 257.5-258.5 °C (dec.). while introduction of the sample at 230 °C gave a melting range of of 265-266.5 °C (dec.).

Preparation of 1-[(1,2-Dimethyl-3,4-dihydro-3H-4-oxo-5-pyrimidinio)methyl]-3-methylimidazolium Diperchlorate (65). A suspension of 1-[(4-amino-1,2-dimethyl-5-pyrimidinio)-methyl]-3-methylimidazolium diperchlorate (1.00 g, 2.39 mmoL) in 6 M hydrochloric acid (10 mL) was heated at 100 °C for 18 h. The solvent was removed under reduced pressure and the residue was extracted with acetonitrile (4 x 10 mL). Evaporation of acetonitrile under reduced pressure produced a hygroscopic solid. Recrystallization from methanol produced 0.300 g (30%, 0.716 mmoL) of colorless needles, mp 202-204 °C (dec.). A sample for analysis was recrystallized first from 90% ethanol and then methanol followed by vacuum

drying over magnesium perchlorate at 100 °C for 5 h, mp 203-205 °C (dec.): ^{1}H NMR (c. 0.24 M, Me_2SO-d_6, Me_4Si) δ2.67 (2'-CH_3), 3.77, 3.90 (N_1'CH_3, N_3CH_3), 5.23 (CH_2), 7.73, 7.77 (H_4, H_5), 8.40 (H_6'), 9.00 (NH, H_2O), 9.20 (H_2). Anal. Calcd for $C_{11}H_{16}N_4Cl_2O_9$ (419.2): C, 31.52; H, 3.85; N, 13.37. Found: C, 31.59; H, 3.88; N, 13.30.

Preparation of 1,2-Dimethyl-5-thiophenoxymethyl-4-pyrimidone (66). A suspension of 4-amino-1,2-dimethyl-5-thiophenoxymethylpyrimidinium perchlorate (0.500 g, 1.45 mmoL) in 6 M HCl (3.0 mL, 18 mmoL) was sealed in a 5 mL glass ampoule and heated at 100 °C for 18 h. The ampoule was cooled to ambient temperature and opened. The solution was made basic to litmus by addition of 5 M sodium hydroxide (4.0 mL, 20 mmoL). The basic solution was extracted with chloroform (4 x 5 mL) and the extract washed first with 1 M sodium carbonate (10 mL) and then with water (10 mL). Following drying over calcium sulfate the solvent was removed under reduced pressure to give 0.334 g (1.36 mmoL, 94%) of a white powder, mp 161.5-163 °C. Recrystallization from acetone yielded 0.317 g (1.29 mmoL, 89%) of white crystals, mp 162-163.5 °C. A sample for analysis was prepared by further recrystallization from acetone and vacuum drying at ambient temperature over magnesium perchlorate for 12 h, mp 162-163.5 °C: ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.32 (2-CH_3), 3.48 (NCH_3), 3.84 (CH_2, d, $J_{CH_2,H_6} \cong 1$ Hz), 7.29 (aromatic mult.), 7.64 (H_6, t, $J_{CH_2,H_6} \cong 1$ Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ21.4 (2-CH_3), 29.6 (CH_2), 40.1 (NCH_3),

118.1 (C_5), 125.9, 128.4, 129.0 (C_2', C_6', C_3', C_5', C_4'), 136.0 (C_1'), 143.0 (C_6), 160.4 (C_2), 168.9 (C_4). Anal. Calcd for $C_{13}H_{14}N_2OS$ (246.3): C, 63.39; H, 5.73; N, 11.37. Found: C, 63.34; H, 5.73; N, 11.35.

pH Measurements in Methanol

Materials

4-Picoline (bp 146.5-146.7 °C, lit.[100] 144.9 °C), piperidine (bp 106.0-106.2 °C, lit.[100] 106 °C), and ethylenediamine (bp 116.5-117.0 °C, lit.[100] 116.5 °C) were purified by distillation from sodium. 4-Aminopyridine was recrystallized from acetonitrile (mp 159-160 °C, lit.[100] 158-159 °C). 1,4-Diazabicyclo[2.2.2]octane (Dabco) was used as received. Perchlorate salts were generated by reacting the base with 70% perchloric acid. 4-Picolinium perchlorate (mp 100-101 °C) and 4-aminopyridinium perchlorate (mp 271.5-273 °C) were recrystallized from isobutyl alcohol and dried at 25 and 100 °C respectively under vacuum over magnesium perchlorate. Dabco monoperchlorate (mp 300 °C, dec.) was recrystallized from a 5:3 (v/v) mixture of ethanol and methanol and vacuum dried at 100 °C over magnesium perchlorate. Ethylenediammonium diperchlorate (mp > 310 °C) was recrystallized from ethanol and vacuum dried at 100 °C over magnesium perchlorate. Piperidinium perchlorate (mp 157.5-158.5 °C) was recrystallized from chloroform and vacuum dried at 25 °C over magnesium perchlorate. Salicylic acid was recrystallized from acetonitrile and sublimed at 100 °C, 1 mm Hg (mp 157-160 °C,

lit.[100] 159 °C). Benzyltriethylammonium perchlorate (BTAP) was generated from the bromide by adding it to sodium perchlorate in water; it was recrystallized from water and vacuum dried at 25 °C over magnesium perchlorate (mp 148-150 °C). Sodium perchlorate used for ionic strength purposes was anhydrous and kept at 120 °C in an oven. All solid materials were kept in a dessicator over calcium sulfate until needed. Methanol (Fisher reagent grade, H_2O 0.1%) was either distilled from magnesium[101] or used as received.

Instrumentation

pH measurements were made with a Radiometer PHM64 Research pH meter in conjunction with a Radiometer GK2321C or GK2401B (low alkaline sodium ion error) combination electrode. Electrodes were prepared for use in methanol by draining the electrolyte (aqueous saturated KCl), rinsing the electrode cavity repeatedly with methanol and filling the cavity with methanol saturated with KCl. The electrodes were equilibrated for an hour in methanol prior to use. Prolonged use and storage of an electrode in methanol tends to dehydrate the glass electrode membrane leading to unstable and erratic measurements.[54] When not in use electrodes were therefore stored in water; replacement of the methanolic electrolyte was necessary to counter any change in the electrode medium by water diffusion across the calomel electrode frit.

Buffer Preparation

Buffer solutions were prepared by two methods.

Method I. The acid and base components were individually weighed into a volumetric flask and diluted to the mark with methanol. Buffer concentrations were chosen such that the conjugate acid concentration was 1.00×10^{-2} M while varying the base concentration. These stock solutions were then diluted to an ionic strength of 1.00×10^{-3} M or diluted with the ionic strength maintained at 1.00×10^{-2} M by the further addition of sodium perchlorate or BTAP.

Method II. Individual acid and base stock solutions were prepared in methanol. Buffers of varying acid/base ratios were prepared by pipetting the appropriate amounts of each component into a volumetric flask and diluting to the mark with methanol. The ionic strength of buffers prepared in this manner was 1.00×10^{-3} M as determined by the conjugate acid concentration.

Salicylic acid buffers were made by half neutralization of salicylic acid with methanolic sodium methoxide standardized with potassium hydrogen pthalate.

General Procedure

Exploratory pH measurements were made at ambient temperature using an estimated pH value of 5.20 for a 1/1 pyridine buffer and 8.99 for a 1/1 Dabco buffer to calibrate the pH meter. The ionic strength of these calibrating buffers was maintained at 1.00×10^{-2} M based on the concentration of the respective conjugate acids. pH values

determined using this calibration were intended to show effects of dilution, water sensitivity, ionic strength, etc., rather than the absolute values of pH.

Absolute pH measurements were made by equilibrating buffer solutions, pH standards and the electrode at the appropriate temperature at least 30 min prior to measurement. The pH meter was calibrated by adjusting the calibration control with a 1/1 Dabco buffer to pH 8.99; this value was set as the iso-pH point on the meter. A 1/1 4-picoline buffer was set to read 6.05 with the sensitivity control. The temperature control was set at 25 °C. The same procedure was followed at 40 °C using pH values of 5.82 and 8.75 for 4-picoline and Dabco buffers respectively. The temperature control was set at 40 °C. The ionic strength of the calibrating buffers was maintained at 1.00×10^{-3} M based on the concentration of the respective conjugate acids.

Determination of pKa Values for Piperidine and 4-Aminopyridine in Methanol

The pH values of a series of piperidine/piperidinium perchlorate and 4-aminopyridine/4-aminopyridinium perchlorate buffers of known concentration were measured at 25.0 and 40.0 °C at ionic strengths of 1.00×10^{-2} and 1.00×10^{-3}M. The ionic strength was determined either by the concentration of the conjugate acid or by added 1.00×10^{-2} M sodium perchlorate or BTAP. Several pH readings of each solution were made; the high and low values are recorded in Tables 5 - 18. After converting pH to hydrogen ion concentration,

Ka values were calculated using the high and low hydrogen ion concentrations, known buffer ratios, and Equation 10. All Ka values for a buffer series at a particular temperature were averaged to give an average Ka, pKa, and standard deviation. Resulting pKa values are recorded in Tables 19-20.

Equation 10 $$Ka = \frac{[H^+][B]}{[BH^+]}$$

Kinetics

Substitution Reactions of Phenol and Pyridine Derivatives of 1'-Methylthiaminium Diperchlorate in Methanolic Piperidine and 4-Aminopyridine Buffers. The synthesis of 4-amino-1,2-dimethyl-5-(p-nitrophenoxymethyl)pyrimidinium perchlorate (NMePmOØpNO$_2$, 7) and 4-amino,1,2-dimethyl-5-(p-cyanophenoxymethyl) pyrimidinium perchlorate (NMePmOØpCN, 8) are described by Zoltewicz.[88] The preparation of the corresponding m-chlorophenoxy, phenoxy, 4-aminopyridinium and piperidino derivatives [NMePmOØmCl (9), NMePmOØH (10), NMePm4AmPy (12), and NMePmPip (56)] are described in the synthetic section of this work. All substrates were recrystallized and vacuum dried before use. Substrate solutions were prepared by weighing into a volumetric flask and diluting to the mark with acetonitrile. Substrate stock solutions were typically 1.00 x 10^{-2} M. Materials and preparation of buffer solutions are described in the pH measurement section of this work.

Kinetic measurements were obtained on a Cary 17-D or Perkin-Elmer 330 spectrophotometer. Multiple sample measurements on the Cary 17-D were made with a 5-cell rotating sample

changing accessory. The cell temperature of the Cary 17-D was maintained at 40.0 or 25.0 °C with a Lauda K-2/R circulating temperature bath. Cell temperature in the Perkin-Elmer 330 was maintained internally with a thermoelectric digital temperature controller. The Lauda bath temperature was determined with an NBS thermometer and the cell temperature with a thermister coupled to a digital read-out. The thermister was calibrated at the bath temperature and the ice-water triple point. Typically a 1.0 °C drop in temperature was noted between the bath and the cell. Reported kinetic temperatures are cell temperatures. Standard 10 mm cuvets with teflon plugs were used for reaction times not exceeding two days. For prolonged kinetic runs, cuvets were designed with a length of quartz tubing which could be sealed with a torch.

A standard procedure was followed for all kinetic runs. Buffer solution (3.00 mL) was delivered by pipet to the cuvet followed by temperature equilibration for a minimum of 30 min. The reaction was initiated by addition of substrate solution (2 to 50 μL) by an appropriate microliter syringe. The wavelengths in nanometers for kinetic analysis were as follows: NMePmOØpNO$_2$, 390 or 400; NMePmOØpCN, 272; NMePmOØmCl, 285; NMePmOØH, 280; NMePm4AmPy, 275. A slight deviation from the general procedure was made when using flame-sealed cells. Both buffer and substrate were added to the cell at ambient temperature and the cell was sealed. The cell was placed in the thermostated cell holder at 40.0 °C and measurement began immediately. Due to the long

reaction times, 2 to 5 days for NMePmOØmCl and NMePmOØH, the time required to reach thermal equilibrium was negligible. Rate data were processed according to standard first-order treatments. The pseudo first-order rate constants, k_ψ, were converted to second-order rate constants, k_2, by dividing by the methoxide ion concentration of the kinetic run, Eq. 20. The concentration of the methoxide ion was determined from the pKa of the buffer and the buffer ratio for the runs, Eqs. 12-14.

$$k_2 = k / [\text{methoxide}] \qquad \text{Equation 20}$$

$$pH = pKa + \log [B]/[BH^+] \qquad \text{Equation 12}$$

$$pOCH_3 = pKs_{(MeOH)} - pH \qquad \text{Equation 13}$$

$$[\text{methoxide}] = 10^{-pOCH_3} \qquad \text{Equation 14}$$

To determine the identity of the products of substitution by piperidine a methanolic piperidine solution (0.20 M, 10 mL) was thermostated in a bath at 41.0 °C and a second solution (0.20 M, 3.00 mL) was thermostated in the spectrophotometer at 40.0 °C. To the bath-equilibrated solution was added NMePmOØpNO$_2$ (50 mg, 1.3 x 10^{-4} moles) giving a 0.013 M substrate solution. Quickly after mixing, a 10 µL aliquot was transferred to the cuvet yielding a substrate concentration of 4.3 x 10^{-5} M. The ensuing reaction was monitored at 400 nm for the appearance of p-nitrophenolate anion (PNP^-). After an approximate five half-lives (3 min) as determined by the UV kinetic run, the main reaction flask was cooled in a dry-ice/acetone bath and methanol removed under vacuum. The NMR spectrum

of the reaction products was taken in DMSO-d_6. The chemical shifts of the products are recorded in Table 47.

The procedure for product determination with 4-aminopyridine is described in the next section.

Substitution Reactions of $NMePmO\emptyset\underline{p}NO_2$ by 4-Aminopyridine in Methanol at 25 °C. Stock solutions of $NMePmO\emptyset pNO_2$ (6.00×10^{-5}, 2.02×10^{-5} M) were prepared by dilution of the corresponding 10^{-3} M parent solutions which were prepared by weight and diluted to the mark with methanol in a volumetric flask. Two stock solutions of 4-aminopyridine (0.999, 0.0999 M) were prepared, the former by weight and the latter by dilution. Samples for kinetic analysis were prepared by pipetting 3.00 mL of substrate solution into a cuvet followed by the appropriate volume of 4-aminopyridine solution (6 to 400 μL) delivered by microliter syringes. The course of the reaction was monitored by following the appearance of p-nitrophenolate anion (PNP^-) at 390 nm using a Cary 17-D spectrophotometer. Multiple sample analysis was accomplished utilizing the rotating cell accessory. The temperature was ambient lab temperature, 25 ± 1 °C. The kinetic data were analyzed by standard first-order treatments.

The substitution products were identified by performing a similar kinetic experiment at NMR concentrations. In an NMR tube were placed $NMePmO\emptyset\underline{p}NO_2$ (0.0506 g, 1.35×10^{-4} moles) and 4-aminopyridine (0.0706 g, 7.50×10^{-4} moles). Methanol was added to give a volume of 0.50 mL (by comparison of the height of the liquid level with that of another in an

NMR tube filled with 0.50 mL of methanol was used as a measure of the total volume). The tube was sealed with a torch and heated at 55 °C. The reaction was monitored by following the production of the aromatic signals assigned to PNP^- and disappearance of those assigned to $NMePmO\emptyset pNO_2$. The reaction reached completion after 60 min. The methanol then was removed under reduced pressure and replaced with D_2O and the contents gently warmed to effect solution. The NMR of the reaction mixture clearly showed a single resonance for the $N\text{-}CH_3$ and CH_2 groups of product but the $C_2\text{-}CH_3$ group had undergone H/D exchange. Also present were signals due to excess 4-aminopyridine and PNP^-. The signals of product were verified as belonging to the 4-aminopyridine substitution product by addition of authentic compound synthesized from 1b and 4-aminopyridine in methanol.

Substitution Reactions of $NMePmO\emptyset pNO_2$ by Azide Ion in Methanol at 25 °C. Stock solutions of substrate (6.11×10^{-3}, 6.14×10^{-3} M) were prepared by weight and diluted to the mark with methanol in volumetric flasks. Stock solutions of sodium azide (0.167, 0.120 M) were similarly prepared. Samples for kinetic analysis were prepared by syringing into a cuvet a constant volume of substrate, 30 μL for one series of reactions and 100 μL for a second series. Various volumes of sodium azide solution were delivered to the cuvet via pipet thereby varying the total azide concentration. The total volume of the cuvet was adjusted to 3.00 mL by addition

Table 47. Proton Chemical Shifts of Reactants and Products for the Substitution of $NMePmO\emptyset pNO_2$ (7) by Piperidine in Methanol.

Compound	$NMePmO\emptyset pNO_2$		PNP^-		NMePmPip		Piperidine	
Proton	Lit.[a]	Found	Lit.[b]	Found	Lit.[c]	Found	Lit.[d]	Found
H_3', H_4', H_5' (piperidine)	——				1.45	1.47	1.50	1.47
H_2', H_6' (piperidine)	——				2.36	2.33	2.74	2.77
2-CH_3	2.65	——			2.55	2.57		
$NMePmCH_2$	5.12	——			3.36	3.33		
NCH_3	3.87	——			3.78	3.77		
H_6'	8.53	——			8.15	8.16		
H_3', H_5' (phenol)	8.29	——	b	7.80				
H_2', H_6' (phenol)	7.29	——	b	6.20				

[a] Prepared by J. A. Zoltewicz (85).
[b] Chemical shifts of phenolates are considerably upfield of phenols but vary with solvent and pH (102).
[c] Prepared by T. D. Baugh (this work).
[d] Ref. 102.

of methanol with a pipet. Kinetic runs were performed as previously described for substitution by 4-aminopyridine. Kinetic data were analyzed by standard first-order treatments.

Product analysis was based on the conversion of NMePmTh to the azide product (NMePmAz) in methanol. The yield was quantitative by NMR; the isolated yield was 38%.

Substitution Reactions of Thiamin (1a, PmTh) and 1-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]pyridinium Chloride (11, PmPy) in Methanol--Materials. Thiamin hydrochloride (Sigma) and the hydrochloride salt of 11[30] were vacuum-dried over magnesium perchlorate for 24 h and stored in a vacuum dessicator. Aniline and triethylamine were distilled before use, 1,4-Diazabicyclo[2.2.2]octane (Dabco) was recrystallized from cyclohexane. 4-Aminopyridine was recrystallized from benzene. Both Dabco and 4-aminopyridine were stored in a dessicator. Benzyltriethylammonium perchlorate (BTAP) was prepared as previously described. Sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TSP) was used as received from Merck, Sharp, and Dohme. Reagent grade methanol (Fisher) was refluxed over magnesium turnings and distilled onto 3A molecular sieves. The sieves were activated by heating at 300 °C for 24 h and stored at 110 °C. All glassware was oven-dried at 110 °C for a minimum of 12 h prior to a kinetic run. The substitution products of 1a and 11 with pyridine, aniline, and 4-aminopyridine were prepared as follows. The preparation of substitution products of 1a and 11 with Dabco and triethylamine will be discussed later.

Preparation of 1-[(2-Methyl-4-amino-5-pyrimidinyl)-methyl]pyridinium Chloride Hydrochloride, 11. The title compound was synthesized according to the method of Shimahara, Nakajima and Hirano.[30] Thiamin chloride hydrochloride (2.00 g, 5.93 mmoL), pyridine (2.00 g, 25.3 mmoL) and methanol (10 mL) were sealed in a screw-cap vial and heated at 100 °C for 4 h. The solvent was removed under reduced pressure and the resulting semi-solid extracted with ethyl ether (8 x 10 mL). The remaining solid (m.p. 249-253 °C, dec.) was recrystallized from 95% ethanol to give 0.380 g of white product, m.p. 256-258 °C (dec.). The mother liquor was concentrated to give a second crop, 0.592 g, of crystals, m.p. 256-258 °C (dec.), (258 °C, lit.). The combined yield was 0.972 g (60%, 3.56 mmoL) of product. Analysis by NMR revealed a small amount of thiamin chloride hydrochloride (5%) remaining in the product. ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.56 (2'-CH_3), 3.88 (NH_2, H_2O), 6.01 (CH_2), 8.19 ($H_{3,5}$, dd, $J_{2,3} \cong J_{3,4}$ = 6 Hz), 8.67 (H_6', s, H_4, t, J ≅ 6 Hz), 9.23 (H_2, H_6, d, J ≅ 6 Hz).

Preparation of N-[(2-Methyl-4-amino-5-pyrimidinyl)methyl]-anilinium Hydrochloride, 23. The title compound was synthesized according to the method of M. Matsukawa and S. Yurugi in aqueous bisulfite medium.[16]

Thiamin chloride hydrochloride (5.00 g, 14.8 mmoL) and aniline (2.94 mL, 3.00 g, 32.2 mmoL) were dissolved in a mixture of ethanol (10 mL) and water (50 mL). To the solution was added 1% sodium bisulfite (10 mL) and the solution was allowed to stand for 16 h. The resulting solid was

collected by filtration giving 2.33 g of a white solid, mp 110-116 °C. Refrigeration of the mother liquor produced 0.63 g of product, mp 115-117 °C. The combined yield, 2.96 g (80%, 11.8 mmoL) was recrystallized from 0.1 M HCl to give 2.50 g (67%, 9.97 mmoL) of a greenish-white solid, mp 114-116 °C (114-116 °C, lit.). ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.50 (2'-CH_3), 3.63 [NH_2 or (NH), H_2O], 4.17 (CH_2), 6.67 (H_2, H_4, H_6), 7.13 (H_3, H_5), 8.10 (H_6'), 8.93 [NH_2 or (NH)].

Preparation of 1-[4-Amino-2-methyl-5-pyrimidinyl)methyl]-4-aminopyridinium Chloride Hydrochloride, 25. Thiamin chloride hydrochloride (5.00 g, 14.8 mmoL), oven-dried at 110 °C for 14 h, and 4-aminopyridine (4.19 g, 44.7 mmoL), recrystallized from benzene and vacuum-dried over magnesium perchlorate, were placed in a heavy-walled glass ampoule. Methanol (25 mL), distilled from magnesium, was added and the ampoule was flame-sealed. The solution was heated at 100 °C for 4 h. The solvent was removed under reduced pressure to give a yellowish semi-solid. The semi-solid was taken up in water (10 mL) and made basic with 1 M sodium hydroxide (20 mL). The basic solution was extracted with ethyl acetate (5 x 10 mL) to remove the liberated thiazole and excess 4-aminopyridine. The aqueous solution was acidified with concentrated hydrochloric acid (10 mL) and the solvent removed under reduced pressure. The resulting solid was extracted with hot 95% ethanol (3 x 20 mL) to separate the organic compounds from sodium chloride. The solvent was again evaporated under reduced pressure and the remaining solid recrystallized from 95% ethanol/acetone.

The yield of product was 0.869 g (20%, 3.00 mmoL) of colorless needles; mp 278-282 °C (dec.): ^{1}H NMR (D_2O, TSP) δ2.70 (2'-CH_3), 5.47 (CH_2), 7.05 (H_3, H_5, d, $J_{2,3}$ = 7 Hz), 8.15 (H_2, H_6, d, $J_{2,3}$ = 7 Hz), 8.12 (H_6'); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ25.3 (2'-CH_3), 53.7 (CH_2), 107.1 (C_5'), 109.5 (C_3, C_5), 142.4 (C_2, C_6), 157.2 (C_6'), 158.8 (C_4), 161.6, 167.7 (C_2', C_4').

Repeated attempts to synthesize the compound again met with failure. Without drying the reagents, massive degradation occurs. Attempts using thiamin perchlorate·$HClO_4$ resulted in the heated solution turning black.

Kinetics

Samples for kinetic runs were prepared by weighing the substrate and nucleophile into an NMR tube. Methanol was added to the tube in a drybox or glove-bag flushed with nitrogen. The solution volume was adjusted to 0.50 mL by comparison with the liquid level in another NMR tube to which 0.50 mL of methanol had been added via syringe. Two or three molecular sieve pellets were added to the solution and the tube was then sealed with a flame. The time-zero NMR spectrum was taken and the tube placed in a water-bath maintained at 71.5 °C with a Lauda K2/R temperature controller. The progress of the reaction was followed by periodically recording the NMR spectrum. Reactions were followed for at least 1.5 half-lives, some up to 4 half-lives.

The reaction of 1a with nucleophiles was followed by monitoring the disappearance of the H-2 signal for the

thiazolium group of 1a and the appearance of the H-2 signal of the liberated thiazole, 3. The extent of formation of 3 was determined by Equation 44 where $\bar{A}$ denotes the average area of the NMR signal from at least four integrations.

$$\% \ 3 = \frac{\bar{A} \text{ H-2 } (3)}{\bar{A} \text{ H-2 } (1a) + \bar{A} \text{ H-2 } (3)} \times 100 \qquad \text{Equation 44}$$

The amount in 1a remaining was calculated according to Equation 45.

$$[1a] = [1a]_o - [1a]_o \times \% \ 3 \times 10^{-2} \qquad \text{Equation 45}$$

Attempts were made to fit the disappearance of 1a to both first-and second-order kinetics using standard equations. For the second-order treatment the reaction was assumed to be first-order in 1a and first-order in nucleophile. Rate constants determined in this manner are denoted as k_ψ, and k_2' for first- and second-order treatments respectively. The conditions and rate constants for the substitution of 1a are reported in Table 34. The reactions of 11 with nucleophiles were followed by monitoring the disappearance of the H-2,6 and H-4 signals of 11 and the appearance of the H-2,6 signals of the liberated pyridine, 6. The H-2,6 signals of 11 appear as a multiplet at 9.10 ppm and shift upfield to 8.60 ppm as 11 releases 6. The H-4 signal of 11, however, appears as a multiplet at 8.75 ppm and overlaps the H-2,6 signals of 6. The overlap was accounted for in the following manner. The area of the H-2,6 protons of 6 was calculated by subtracting the area

of the H-4 proton of 11 from the total area of the overlap region. The area of the H-4 proton of 11 is equal to one-half the area of the H-2,6 protons of 11. The extent of formation of 6 was then determined by Equation 46 where $\bar{A}$ denotes the average integrated area of at least four determinations.

$$\% \underline{6} = \frac{\bar{A}\,[\text{h-2,6 } (\underline{6}) + \text{H-4 } (\underline{11})] - 0.5\,\bar{A}\,[\text{H-2,6 } (\underline{11})]}{\bar{A}\,[\text{H-2,6 } (6) + \text{H-4 } (\underline{11})] + 0.5\,\bar{A}\,[\text{H-2,6 } (\underline{11})]} \times 100$$

Equation 46

The amount of 11 remaining was determined by Equation 47

$$[\underline{11}] = [\underline{11}]_o - [\underline{11}]_o \times \% \underline{6} \times 10^{-2} \qquad \text{Equation 47}$$

Rate constants, k_ψ and k_2', obtained as described for 1a, and conditions for substitution of 11 are reported in Table 35.

Concentrations of nucleophiles and substrates reported in Tables 34 and 35 were corrected for thermal expansion of methanol. Concentrations determined at ambient temperature, assumed to be 25 °C, decrease by 5.7%. This correction factor was determined by Equation 48 where d_{25} and $d_{71.5}$ are the densities of methanol at the indicated temperatures, 0.7870 and 0.7444 g/mL.[103]

$$[\ ]_{71.5\ °C} = [\ ]_{25\ °C} \times \frac{d_{71.5\ °C}}{d_{25\ °C}} \qquad \text{Equation 48}$$

A mass balance for the conversion of 1a and 6-d_5 to 11-d_5 and 3, was determined by comparing the area due to the aromatic protons of 1,3,5-trimethylbenzene, 6.78 ppm, to the area of the thiazole H-2 signal of 1a and 3, 9.5-9.8

and 8.8 ppm respectively. The total amount of thiazole, 1a + 3, was compared to the standard for each kinetic point in runs 1 and 2, Table 34. The mass balance observed in run 1 was 102±5% over 1.7 half-lives. Similar results were obtained in run 2, 95%±7%, over 1.4 half-lives. The conversion of 1a to 3 is therefore the major process for the reaction of 1a with 6-d_5.

The appearance of the methylene signal of product 11-d_5, 6.02 ppm, was compared to the appearance of the H-2 signal of 3 periodically during the course of the reaction stated above. In all cases, the amount of 11-d_5 was equal to the amount of 3 as determined by area integration. Formation of 11-d_5 is therefore correlated with the liberation of 3 as the major pathway. Mass balance for reactions of 1a with 4-aminopyridine, aniline, and Dabco was not measured directly but are assumed to be adequately modeled by reactions of 11 with these nucleophiles as described below.

Mass balances for reactions in methanol were investigated in separate studies at 100 °C. In two separate studies the signals attributable to 11 and to the substitution products were integrated with respect to internal standards, benzyltriethylammonium perchlorate (BTAP) and sodium 3-trimethylsilylpropionate-2,2,3,3,-d_4 (TSP). Solutions of 11, nucleophile, and standard in methanol were sealed into NMR tubes. The ratio of 11 to standard was obtained by integration of H-2,6, 9.09 ppm, or the methylene group of 11 and the methyl triplet, 1.27 ppm, or aromatic signal, 7.63 ppm, of BTAP or methyl signal of TSP, 0 ppm. Reactions were

run for five hours at 100 °C. The ratios of product to standard were obtained by integration of product methylene or pyrimidine H-6 signals and the appropriate signal of the standard. To unmask signals hidden by the reaction solvent, the solvent was removed under reduced pressure. The substitution products are expected to be quaternary amines and hence, non-volatile. Product to standard ratios were redetermined in a 3:2 (v/v) mixture of Me_2SO-d_6:D_2O; D_2O served to exchange obstructing amino group signals.

Satisfactory mass balances were obtained for aniline and Dabco nucleophiles. In each case complete reaction of 11 was observed as evidenced by the disappearance of the methylene signal of 11 at δ6.0. Aniline and 11 reacted quantitatively to produce the expected substitution product; proof of structure is deferred to later discussion. Mass balance for this reaction was 100% based on the appearance of the new methylene signal at 4.23 ppm referenced to either of the internal standards. Dabco and 11 also reacted quantitatively with a mass balance of 90% referenced to BTAP and 104% referenced to TSP. Mass balance was based on the appearance of the new pyrimidine H-6 proton at 8.35 ppm.

A mass balance for the reaction of 11 with 4-aminopyridine and triethylamine was not obtainable. Paramagnetic contamination of the 4-aminopyridine caused line broadening resulting in severe signal overlap, which interfered with mass determination. Triethylamine did not completely react with 11 under the experimental conditions. The extent of conversion was not determined due to extreme overlapping of

signals. Spectrometer resolution was much poorer in this case than for kinetic runs.

The substitution product of the reaction of 1a with pyridine, 6, was identified as 11. Product chemical shifts are identical to those of authentic 11, Table 48. On a synthetic scale, 11 was isolated in 60% yield as previously discussed.

The substitution product of 1a with aniline was shown to be 23 by comparison of the chemical shifts of the product in kinetic runs with those of authentic 23, Table 49. The reaction of 11 with aniline in mass balance experiments also produced 23. Addition of authentic 23 to the reaction mixture in Me_2SO-d_6/D_2O confirmed the identity of the product.

Reactions of 1a and 11 with Dabco give the same substitution product as evidenced from the observed chemical shifts in kinetic and mass balance runs, Table 50. The upfield shift of the methylene group is consistent with alkylation by aliphatic nitrogen compounds; see the shifts of the 1'-methylated analog. The possibility of formation of the methoxymethyl derivative is unlikely based on the chemical shifts for this compound, Table 53. Quaternization of N-1 for methoxymethyl results in substantial shifts for the 2-CH_3 and H-6 signals. Since excess Dabco was used, the chemical shifts of the Dabco substitution product should be that of the pyrimidine free base because Dabco, being more basic, deprotonates the pyrimidine ring to generate free base. Based on these considerations it is probable that the substitution product is that of amine substitution.

The reactions of 1a and 11 with 4-aminopyridine yield the same substitution product based on the chemical shift data of Table 51. The chemical shifts of the product are the same as those for the isolated derivative described earlier and are analogous to those of the 1'-methyl derivative, 12. The substitution product is postulated to be 1-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-4-aminopyridinium chloride, 25.

The structure of the product of triethylamine substitution of 11 is not clear. Kinetic runs with 11-d_5 show the formation of a new pyrimidine H-6 proton at δ8.30, Table 52. In mass balance experiments using proteo 11, the aromatic region is obscured by the presence of both quaternized and free pyridine. The spectrum in Me_2SO-d_6:D_2O however shows a new methylene group at 5.69, Table 52, and the appearance of quaternized triethylamine. The methyl shift of triethylamine is confirmed by comparison with the methyl shift of BTAP, which appears at δ1.49. The substitution product is tentatively identified as 4-amino-2-methyl-5-triethylammoniomethylpyrimidine.

Table 48. Proton Chemical Shifts of the Pyridine Substitution Product, 11, and Its 1'-Methyl Analog, 53.

	11		53
Signal	CH_3OH[a]	Me_2SO-d_6[b]	Me_2SO-d_6
2'CH_3			2.63
CH_2	5.97	6.02	5.75
H_6'	8.44	8.40	8.50
$H_{3,5}$	---[c]	8.22	8.20
H_4	---[c]	8.76	8.70
$H_{2,6}$	---[c]	9.09	9.05
1'-CH_3			3.92

[a]Shifts found in kinetic runs.
[b]Authentic product.
[c]Deuterated pyridine.

Table 49. Proton Chemical Shifts of the Aniline Substitution Product, 23, and Its 1'-Methyl Analog, 67.

	23				67
Signal	CH_3OH		Me_2SO-d_6		Me_2SO-d_6
	a	b	c	b	
2'CH_3	2.49	2.49	2.43	2.40	2.60
CH_2	4.22	4.19	4.32	4.23	4.17
H-6'	7.96	7.92	8.10	8.07	8.27
H-2,4,6	---[e]	---[e]	---[e]	6.70	6.70
H-3,5	---[e]	---[e]	---[e]	7.20	7.20
1'-CH_3					

[a]Shifts found for kinetic runs with 1a.
[b]Shifts found for mass balances runs with 11.
[c]Product from mass balance determination.
[d]Authentic product.
[e]Signals obscured by excess aniline.

Table 50. Proton Chemical Shifts of the Dabco Substitution Product, 24, and Its 1'-Methyl Analog, 55.

Signal	24			55
	MeOH		Me_2SO-d_6	Me_2SO-d_6
	a	b	b	
$2'CH_3$	2.50	2.46	2.47	2.63
$2'CH_2$ (Dabco)	3.4	3.4	3.4	3.70[c]
$3'CH_2$ (Dabco)	3.4	3.4	3.4	3.70[c]
CH_2	4.71	4.73	4.67	4.67
H-6	8.28	8.21	8.27	8.47

[a]Shifts found for kinetic runs of 1a.
[b]Shifts found for kinetic and mass balance runs of 11.
[c]The Dabco moiety appears as the dication.

Table 51. Proton Chemical Shifts of the 4-Aminopyridine Substitution Product, 25, and Its 1'-Methyl Analog, 12.

Signal	25			12
	MeOH	Me_2SO-d_6		Me_2SO-d_6
	a	b	c	
$2'CH_2$	2.47	2.47	2.70	2.60
CH_2	5.42	5.27	5.47	5.23
$H_{3,5}$	---[d]	6.93	7.05	6.85
$H_{2,6}$	---[d]	8.08	8.15	8.15
H-6'	8.31	8.17	8.12	8.31

[a]Shifts found for kinetic runs with 1a.
[b]Shifts found for mass balance runs with 11.
[c]Authentic product as the hydrochloride salt.
[d]Shifts obscured by excess 4-aminopyridine.

Table 52. Proton Chemical Shifts for the Substitution Product of 11 and Triethylamine, 26.

Signal	MeOH[a]
2-CH_3	2.45
2'CH_3	1.49[b]
1'CH_2	3.50[b]
5-CH_2	4.69
H_6	8.30

[a]Shifts found for kinetic runs with 11.
[b]Chemical shifts of triethylamine in methanol are 1.24 (CH_3) and 2.97 (CH_2N).

Table 53. Proton Chemical Shifts for 4-Amino-2-methyl-5-methoxymethylpyrimidine 68 and Its 1'-Methyl Analog 69 in Me_2SO-d_6.[a]

Signal	68	69
2-CH_3	2.33	2.59
OCH_3	3.26	3.35
NCH_3	---	3.83
CH_2	4.26	4.32
H-6	7.95	8.35

[a]Ref. 104.

Cyclization of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)-methyl]-2-aminopyridinium Diperchlorate 33 to 2,3-Dimethylpyrichrominium Perchlorate 34. Sample Preparation. Evaporation under reduced pressure of the mother liquors generated during the purification of 33 produced a 50/50 mixture of 33 and 34. The perchlorate salts were converted to the chlorides by stirring the mixture in a solution of potassium chloride (3 mL of saturated potassium chloride and 2 mL of water) for 30 min followed by filtration of potassium perchlorate. The resulting solution of chloride salts was evaporated to dryness under reduced pressure. The mixture of salts was stirred in methanol (2 mL) for 30 min and the methanolic solution of 33 and 34 was separated from the inorganic salts by filtration to yield the stock solution for the cyclization reactions. Cyclization Reactions. Aliquots of stock solution (0.50 mL) were syringed into each of three NMR tubes. The control tube was sealed with no added base. To the second tube was added 2,4,6-trimethylpyridine and triethylamine was added to the third. Both tubes were then sealed. The tubes were allowed to stand at ambient temperature for 90 min followed by heating at 71.5 °C. Production of 34 was monitored by integrating the methylene signal against the carbon-13 satellite of methanol. The methylene group of 34 lies on the shoulder of the OH resonance of methanol.

Measurement of Interatomic Proton Distances of the Isomeric Forms of 2,3-Dimethylthiachrominium Perchlorate, 36, and the Conjugate Acid of 2,3,9-Trimethylpyrichrominium Perchlorate, 37, Based on Dreiding Models. Approximate interatomic proton distances of the isomeric forms of 36 and 37 were measured using Dreiding models. Although the molecules exhibit some bending about the methylene group common to all isomers, measurements were based on a flat form in which the two protons of the methylene group exactly bisect the plane of the molecule being measured. The flat form should approximate the time averaged solution conformation of the molecule being measured. Distances were measured from the centers of the hydrogen nuclei. A 90° sulfur bond angle was incorporated into the isomers of 2,3-dimethylthiachrominium perchlorate based on the sulfur bond angle found for thiochrome by x-ray analysis.[105]

Proton-Proton Nuclear Overhauser Enhancements for 2,3-Dimethylthiachrominium Perchlorate, 36, and the Conjugate Acid of 2,3,9-Trimethylpyrichrominium Perchlorate, 37. Sample Preparation. 36 was recrystallized three times from water. 37 was recrystallized two times from 0.1 M perchloric acid. 36, 37, and 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were vacuum dried at 100 °C over magnesium perchlorate for 2 h and kept in a dessicator over P_2O_5.

All glassware used in sample preparation was soaked for 24 h in 0.1 M disodium ethylenediaminetetraacetic acid to complex paramagnetic ions, rinsed with D_2O and dried at 110 °C for 12 h. A 5% w/v sample was prepared using 50 mg of

36 or 37, 10 mg of DSS in 1.00 mL of Me_2SO-d_6 (Aldrich, 99.5 atom %). The solution was filtered into an NMR tube through celite which had been washed with D_2O and dried at 110 °C for 12 h. The sample was degassed through five freeze-thaw-pump cycles and the tube sealed under vacuum. Data Acquisition. NMR spectra were obtained on a Jeol FX-100 spectrometer operating at 99.6 MHz, frequency locked on solvent deuterium at an ambient probe temperature of approximately 25 °C. The homonuclear decoupler frequency for selective irradiation of the methylene group in 36 and 37 was set by observing the collapse of benzylic coupling between H_4 and the irradiated methylene group. The decoupler power was set as low as possible while retaining maximum signal intensity of H_4. Each spectrum was the result of 40 transients taken with a tip angle of 86°, acquisition time of 3.41 sec., and a delay between transients of 45 sec. Data were recorded when the methylene group was irradiated and also when the irradiation was moved off resonance 60 to 70 Hz. A cycle of irradiation was performed as follows: on CH_2; off resonance to high field; on CH_2; off resonance to low field the cycle was repeated five times to give a total of twenty spectra. Repetition of the irradiation cycle was employed to offset any changes in spectrometer performance with time. The off-resonance irradiation was alternated between high and low field with respect to the CH_2 group in order to check the possibility of spectrum perturbation due to the position of the decoupler frequency. Treatment of Data. Peak areas for each spectrum

were obtained by integration. The methyl signal of DSS was used as an internal standard. The ten values obtained for each signal while irradiating the methylene group of 36 or 37 were averaged as were the five values for off-resonance high-field irradiation and off-resonance low-field irradiation. For the case of 36 both off-resonance values were averaged. For 37 however, only the low-field off-resonance values could be taken as standard; the high-field irradiation frequency conincided with the NH signal causing enhancements of other protons associated with their proximity to the NH group in the molecule. The low-field irradiation values were used as standards and compared to that resulting from the irradiation of the methylene group and the NH group. Nuclear Overhauser enhancements were calculated according to Eq. 49 where $\overline{X}_1$ is the averaged signal intensity for the on-resonance irradiation case and $\overline{X}_2$ is the averaged signal intensity for the off-resonance irradiated case.

$$\%NOE = \frac{\overline{X}_1 - \overline{X}_2}{\overline{X}_2} \times 100 \qquad \text{Equation 49}$$

A statistical analysis of the data at 95%, 99%, and 99.9% confidence levels was performed and error limits calculated according to the methods described by Mendenhall.[110]

Proton Relaxation Rates (T_1) for 2,3-Dimethylthiachrominium Perchlorate, 36. Sample Preparation. An 8% (w/v) solution of 36 in Me_2SO-d_6 was made according to the procedure used to prepare a sample for NOE determination. Acetonitrile (5 µL/mL solution) was substituted for DSS as an internal standard. Data Accumulation. T_1 data were obtained using

the inversion-recovery method with 90° and 180° tip angles corresponding to 32.5 and 65 μS pulses. The inversion-recovery sequence, 180° pulse-delay (τ)-90° pulse-acquisition-delay (T), was repeated four times per spectrum with a 20 sec delay (T) between cycles. A total of twenty τ values were used ranging from 0.01 to 15.0 sec. Data Processing. T_1 values were determined by plotting ln $(S_\infty - S_\tau/S_\infty)$ against τ and taking the inverse of minus the slope according to Equations 50 and 51.

$$-k\tau = \ln (S_\infty - S_\tau)/S_\infty \qquad \text{Equation 50}$$

$$T_1 = 1/k \qquad \text{Equation 51}$$

The relaxation rate constant is k and τ is the time interval during which a proton relaxes after the 180° pulse. S_∞ is the signal intensity of a proton completely relaxed after the τ interval, and S_τ is the signal intensity of a proton after relaxing for a particular τ interval. Signal intensities were based on peak height measurements. The total intensity of doublet signals was taken as the numerical sum of the intensities of each leg. S_∞ was measured several times throughout the experiment using τ = 15 sec to minimize changes in intensity values due to spectrometer performance with time.

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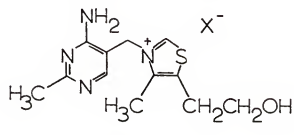
APPENDIX

PREVIOUSLY PUBLISHED INVESTIGATIONS

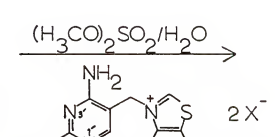
The material contained in this section has been separated from the main text as it has already been published. It consists of two parts. The first, "An Improved Synthesis of 1'-Methylthiaminium Salts," has been published in Synthesis. The second, "New Highly Fluorescent Derivative of Adenosine. Cyclization of Adenosine with 1'-Methylthiaminium Ion," was published in the Journal of Organic Chemistry. Both articles are presented here exactly as they appear in the literature.

AN IMPROVED SYNTHESIS OF 1'-METHYLTHIAMINIUM SALTS*

1'-Methylthiaminium salts 2 promise to be important new substrates for the continuing exploration of the chemistry of thiamin.[1] The N-methyl group is located on that annular nitrogen atom which is favored thermodynamically for protonation.[2,3] Salts 2 are thus useful substitutes for protonated thiamin. Quaternization confers positive charge on the pyrimidine ring just as protonation does, but quaternization has the advantage that the ring acquires positive charge in a pH-independent process.



1



2 $X^- = ClO_4^-, Cl^-$

A preparation of 2 in unstated yield has been reported[4] which involves the mononitrate salt of thiamin being treated with methyl iodide in refluxing methanol. In our hands this preparation proved to be unsatisfactory, largely because massive amounts of degradation took place. The fragmentation is reminiscent of that in which the thiazole ring

*J.A. Zoltewicz and T.D. Baugh, Synthesis, 217 (1980).

is liberated while providing a component containing the pyrimidine ring.[5]

The preparation of 2 can be effected conveniently and in high yield simply by using water as the solvent and dimethyl sulfate as the methylating agent. Quaternization is rapid at room temperature. The addition of one equivalent of dimethyl sulfate converts most of the thiamin 1 to product 2 but several equivalents are added to drive the reaction to completion, N.M.R. spectrometry serving as a useful way to follow the extent of the conversion. The material is readily isolated as its moderately soluble diperchlorate salt (X = ClO_4); it can be converted easily to its more water soluble dichloride (X = Cl). On melting, salts of 2 which have been air-dried show a polymorphism similar to that of thiamin.[6]

The site of methylation is proven by natural abundance ^{15}N-N.M.R., taking advantage of the chemical shifts collected in a careful study used to show that the site of protonation of thiamin is N-1'[2]. As the data in the Table show, the chemical shifts of protonated and methylated thiamin are nearly the same, strongly suggesting that protonation and methylation occur at the same nitrogen atom. Our chemical shifts for protonated thiamin differ only slightly from reported values,[2] differences possibly reflect changes in concentration and solvent.

If N-3' had been the site of quaternization, then markedly different chemical shifts would have been observed. The chemical shifts of thiamin free base reported in the Table may be corrected to estimate shifts for N-3' quaternized

Table. ^{15}N-N.M.R. Chemical Shifts (δ ppm) of Thiamin and Derivatives at Natural Abundance

Ring N	Thiamin Chloride Hydrochloride		1'-Methyl-thiaminium Salt[b]	Thiamin Free Base[a]
	Ref. 2[a]	This work[b]		
Thiazole	130.5	137.4	133.0	134.0
N-3'	164.7	169.9	169.6	161.8
N-1'	208.8	212.2	214.7	135
NH_2	266.8	271.4	273.1	288.0

[a]External HNO_3 is the reference standard at 0 ppm. Ethylene glycol is the solvent (Δ_δ HNO_3-CH_3NO_2 = 0.0 ppm).

[b]External CH_3NO_2 is the standard at 0 ppm. Samples were c = 1 mol/l in 10% D_2O/90% H_2O.

material. Magnitudes of correction factors may be obtained from protonation and N-methylation data.

Protonation of thiamin produces a large change in the chemical shift of only one annular nitrogen atom. As the Table shows protonation results in a shielding of 73.8 ppm for N-1'. By comparison, N-methylation of pyridine leads to a shielding of 111.5 ppm.[7] Thus, if N-3' were quaternized instead of N-1' a signal in the range of 235 to 273 ppm would have been observed. This range is well outside that actually observed for the annular nitrogen atoms of N-methylated thiamin.

Such a high field signal cannot be confused with that for the nearby amino group because only the latter shows large proton coupling. Other annular nitrogen atoms would show only modest changes due to quaternization at N-3'. The

fact that no signal was observed in the high field region being considered serves not only to confirm the structure but also to rule out the presence of significant amounts of isomeric impurity. In further support of the structural assignment is the fact that N-1′ is predicted to be considerably more reactive than N-3′. The prediction is based on kinetic data for model compounds.[8,9,10]

Now that a convenient preparation of salts 2 is available it will be most interesting to learn whether they can serve as a substitute for thiamin in enzymic reactions.

Spectra were taken on a Varian FT-XL-100-15 spectrometer equipped with a Nicolet Multi Observe Nuclei Accessory. The instrument was locked on internal deuterium present in the solvent.

1′-Methylthiaminium Perchlorate (2):

Sodium hydrogen carbonate (62.3 g, 0.741 mol) is slowly added to a stirred solution of thiamin chloride hydrochloride (1·HCl; 250 g, 0.741 mol) in water (700 ml). When neutralization is complete, calcium carbonate (37.08 g, 0.371 mol) is added as an acid scavenger. Dimethyl sulfate (234 g, 176 ml, 1.85 mol) is slowly added to the mixture over a period of 2.5 h. During this time the pH of the mixture is monitored and sodium hydrogen carbonate is added as necessary to maintain the pH at about 6.5. About 38 g of sodium hydrogen carbonate is used. As the reaction proceeds the signal of H-2 starting material shifts from 9.60 ppm to 9.82 ppm. When methylation is complete the mixture is filtered and the solution transferred to a large beaker with

mechanical stirring. To this is added a solution of sodium perchlorate (362.6 g, 2.96 mol) in water (500 ml). The resulting thick mixture is stirred for 4 h, filtered, and the filter cake of product washed with two portions of ice-cold water (2 x 100 ml). The product is then dried under vacuum, 339 g being recovered. Recrystallization of a 100 g portion of crude material from 0.1 molar perchloric acid affords pure product; yield: 80.2 g (77%); m.p. 236-239 °C (dec.).

The material liquifies above 200 °C and resolidifies. Decomposition occurs at a higher temperature and is somewhat variable. A sample for microanalysis was prepared by twice recrystallizing from 0.1 molar perchloric acid. The material then was suspended in deionized water, filtered, and dried for several hours in an evacuated drying pistol containing anhydrone at 100 °C. No significant liquification took place prior to decomposition at 240-241.5 °C.

$C_{13}H_{20}Cl_2N_4O_9S$	calc.	C 32.58	H 4.21	N 11.69
(479.3)	found	32.56	4.23	11.67

A sample of this material was kept open to the room for about two days and reanalyzed; no significant hydration occurred.

Proton chemical shifts for a 0.1 molar sample in DMSO-d_6 (TMS) are substantially the same as those in Ref. 4 except that we find NCH_2, H-6' and H-2 to be shifted 0.09, 0.18, and 0.19 ppm to higher field.

An estimate of the solubility of the diperchlorate salt in water was obtained by stirring the salt in deionized water

at 23±2 °C for 72 h. The mixture was filtered and the density of the saturated solution was found to be 1.0048 g/ml. The concentration of dissolved material is 0.0073 g/ml after correcting for the density of pure water. Hence, the solubility is 0.015 mol/l. Note that warm solutions tend to supersaturate on cooling.

^{13}C-N.M.R. (c 1.0, D_2O; 26 °C, DSS as standard): δ = 13.7 (4-CH_3); 24.0 (2'-CH_3); 31.8 (α-CH_2); 44.9 (NCH_3); 52.2 (NCH_2); 62.8 (β-CH_2); 109.1 (C-5'); 138.8 (C-5); 145.2 (C-4); 152.6 (C-6'); 156.7 (C-2); 164.4, 166.7 ppm (C-2', C-4' not assigned). These shifts are similar to those for thiamin chloride hydrocloride.[11] Off resonance was employed to aid signal assignments.

1'-Methylthiaminium Chloride (2):

1'-Methylthiaminium perchlorate (20.0 g, 0.0418 mol) is stirred for 3 h with saturated potassium chloride solution (27 ml, c 3.74 mol/l). The mixture is filtered, washed twice with saturated potassium chloride solution (2 x 1 ml). The combined aqueous solution is evaporated to dryness under vacuum. The bulk of the solid is dissolved in hot methanol (50 ml) to remove inorganic salts. Methanol is then removed under vacuum to give 1'-methylthiaminium dichloride; yield: 13.8 g (92%); m.p. 245-247.5 °C (dec.). An analytical sample was prepared by recrystallization from 95% ethanol/5% concentrated hydrochloric acid; m.p. 249-251 °C (dec.).

$C_{13}H_{20}Cl_2N_4OS \cdot 0.5\ H_2O$	calc.	C 43.27	H 5.87	N 15.56
(306.3)	found	43.27	5.92	15.49

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NEW HIGHLY FLUORESCENT DERIVATIVE OF ADENOSINE. CYCLIZATION OF ADENOSINE WITH 1'-METHYLTHIAMINIUM ION*

Considerable effort has been expended to convert adenosine (I, Chart I) into fluorescent derivatives. Such conversions not only provide an ultrasensative method of detecting I but also furnish fluorophores which are useful bioprobes.[1]

Successful transformations largely include those which fuse a five-membered ring onto I by incorporating N-1 and the 6-amino group along with a reagent such as chloroacetaldehyde[2,3] or glyoxal.[4] Emphasis now is being placed on the synthesis of new fluorescent derivatives of heteroaromatic components of nucleic acids by annulation to give six-membered rings.[1]

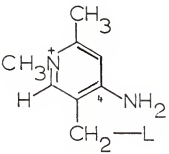
We report the preparation of a novel, highly fluorescent derivative of I. Two heterocyclic rings are fused onto I, both six members, by treatment with 1'-methylthiaminium ion (ii),[5] a derivative of vitamin B_1.

Results and Discussion

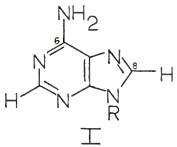
Compounds I and II readily react in refluxing methanol containing 2,4,6-trimethylpyridine catalyst.[6] Proton and ^{13}C NMR show that the product does not contain the thiazole ring (L) from II. In view of the many facile nucleophilic

*J.A. Zoltewicz and T.D. Baugh, J. Org. Chem., 47, 1351 (1982).

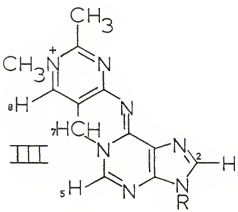
Chart I



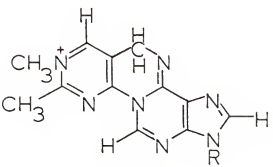
II



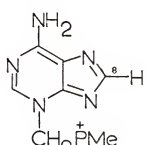
I



III

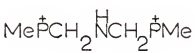


IV

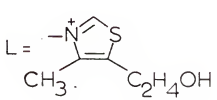


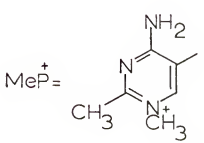
V

R=ribose



VI





substitution reactions which II undergoes,[7] I must be bonded to II at its CH_2 group in place of the thiazole ring. Elemental analyses reveal that the substitution product is cyclic, cyclization proceeding by the loss of an amino group as ammonia. Therefore, the product is likely to have structure III or IV, both containing four fused heterocyclic rings having a total of seven annular nitrogen atoms.

Regioisomers III and IV differ by having the orientations of the two reactants reversed on cyclization. Isomer III has the CH_2 group of II bonded to N-1 of I. One of the two amino groups is incorporated into the new ring, the other is lost as ammonia. Isomer IV has the CH_2 chain attached to the 6-amino group of I; N-1 of I is bonded to position 4 of II in place of its amino substituent.

Differentiation between these two isomers was achieved by means of a nuclear Overhauser effect (NOE) involving aromatic protons. Irradiation of the CH_2 signal is expected to enhance the signals of <u>two</u> adjacent protons in III but only <u>one</u> in IV. Other aromatic protons in IV are located too far away. Our crucial observation in that two signals demonstrate substantial enhancement when the signal due to CH_2 is irradiated. The product is III, a new ring system. A systematic name for it is 9,10-dimethyl-3-ribosyl-3,7-dihydropyrimido[4',5':4,5]pyrimido[2,1-i]purinium perchlorate.[8]

Independent assignment of the three aromatic proton signals to sites in the cyclized product supports structure III and the observed NOE. Warming III in D_2O causes the signal at lowest field to decrease in intensity as a result

of hydrogen-deuterium exchange.[11] Irradiation of the 1'-proton of ribose induces an NOE in this low-field hydrogen signal. Both observations suggest that this signal may be assigned to H-2 which is bonded to the imidazole ring of III. The highest field aromatic proton signal is slightly broadened by coupling with the CH_2 group and is, therefore, H-8 of III, the pyrimidine proton from II.[12] The remaining aromatic hydrogen signal must be associated with H-5, the pyrimidine proton from I. As required by structure III, NOE enhancements due to irradiation of the CH_2 signal are found in the two signals assigned to the two pyrimidine protons. The product must have the cyclopentenoanthracene geometry of III and not the steroid geometry of IV.

The scope of the heterocyclization reaction is indicated by a consideration of the structure of the product from II and adenine, potentially a tetracyclic substance similar to III or IV. However, NMR and elemental analyses clearly show that N-alkylation, not cyclization, is the major pathway. Structure proof reduces to the classical problem, solved now in several ways, of determining the site of N-alkylation of a purine. In a separate experiment, position 8 of adenine was deuterated.[13] This material on N-alkylation with II no longer showed a signal at $\delta 7.8$; therefore, this signal must be associated with H-8 of the product. Of the four possible ring N-alkylated isomers only the N-3 product shows a signal at such high field.[14,15] Our product must be N-3 isomer V. Adenine methylates at the same position.[16]

In keeping with the formation of V, we conclude that III probably forms by alkylation of N-1 of I in the first step followed by cyclization. Had the first step been substitution with the loss of an amino group, both adenosine and adenine would have yielded tetracyclic products by subsequent cyclization.

An excess of II must be employed to achieve high conversions of I. The ammonia liberated in the cyclization step serves as a nucleophile which acts in competition with I and II to give nonfluorescent secondary amine VI having two pyrimidine rings. Amine VI may be independently synthesized from II by using NH_4ClO_4 as a source of low concentrations of ammonia.

The fluorescence properties of III are remarkable. Concentrated solutions show little fluorescence due to self-quenching. A 2.4×10^{-3} M solution in pH 9.2 aqueous buffer shows excitation (323 nm) and emission spectra (429 nm) which are different from those for more dilute samples. Dilution causes excitation and emission peaks to shift position and to increase in intensity initially. A 500-fold dilution shifts the main excitation to longer and the emission to shorter wave lengths. (The excitation and ultraviolet absorption sepctra are quite similar.) Further dilution lowers both intensities; a 1×10^{-7} M solution has its main excitation band at 385 nm and the emission band at 408 nm. We could easily detect the fluorescence of a 5×10^{-10} M sample of III in water in spite of the small Stokes shift, with excitation at the 385-nm maximum and detection at the 408-nm maximum. The Raman band

of water appears as an emission at 440 nm under these conditions and does not interfere. Detection of III by fluorometry appears to be on sensitivity levels comparable to those for other adenosine derivatives.[3,4]

No doubt other substrates can be converted to fluorescent analogues with II. Our fluorescent derivative and synthetic method await exploration.

Experimental Section

Preparation of Fluorescent Derivative III. A suspension of 2.50 g (9.36 mmol) of adenosine, 6.7 g (14 mmol) of 1'-methylthiaminium diperchlorate,[5] 4 mL (30 mmol) of 2,4,6-trimethylpyridine, and 125 mL of methanol was heated at reflux with stirring for 17.25 h. The creamy white filter cake was washed with ethyl acetate to give 3.05 g (5.8 mmol, 62%) of raw product, mp 200-208 °C dec. A sample in D_2O showed the presence of a very small amount of unreacted adenosine (1'-ribose doublet of adenosine is about 10 Hz upfield from that of III) along with a minor amount of secondary amine VI (CCH_3 singlet falls about 8 Hz upfield from that of III). Recrystallization from ethanol-water (product has a tendency to supersaturate) gave the analytical sample (mp 216-219 °C dec) which was dired at room temperature under vacuum: UV (2.39×10^{-5}M in pH 9.2 borate buffer) 206 nm (ε 2.51×10^4), 245 (1.15×10^4), 383 (3.60×10^4), H NMR (D_2O, DSS) δ 8.65, 8.55, 8.3 (broadened, each 1 H), 6.15 (ribose 1'), 5.7 (CH_2, broadened), 5.54-4.9 (ribose), 3.95 (NCH_3), 2.7 (CCH_3); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ163.4, 163.0, 151.2, 148.5 (4 s), 147.5, 144.8, 143.1 (3 d), 124.3, 110.9 (2s),

87.7, 85.5, 74.4, 70.1 (4 d), 61.0 (t, CH_2OH), 46.2 (t, CH_2N), 42.6 (q, NCH_3), 21.9 (q, CCH_3). Anal. Calcd for $C_{17}H_{20}N_7ClO_8 \cdot 2H_2O$: C, 39.12; H, 4.63; N, 18.78. Found: C, 39.09; H, 4.57; N, 18.77. Heating a sample in D_2O at 100 °C for 3 h resulted in at least 80% deuteration of the lowest field aromatic proton and about 50% deuteration of the CCH_3 group.

3-[(4-Amino-1,2-dimethyl-5-pyrimidinio)methyl]adenine Perchlorate (V). A suspension of 0.500 g (3.07 mmol) of adenine, 3.68 g (6.14 mmol) of 1'-methylthiaminium diperchlorate,[5] 2 mL (15 mmol) of 2,4,6-trimethylpyridine, 40 mL of methanol, and 10 mL of dimethyl sulfoxide was heated at reflux for 1 h. Following filtration of the hot mixture and two washings with 10 mL portions of methanol, 1.00 g of product (mp 280-281 °C dec) was collected. Recrystallization from 90 mL of 90% aqueous ethanol gave 0.725 g (2.0 mmol, 64%) of product, mp 286-287 °C dec. (Another mixture heated for 24 h gave the same product.) An analytical sample was prepared by recrystallization from 50% aqueous acetonitrile; mp 286-287 °C dec. It was dried at 100 °C under vacuum over $MgClO_4$: ^1H NMR (Me_2SO-d_6, Me_4Si) δ9.30 (NH), 8.50, 8.44 (H-2, H-6'), 8.13 (NH), 7.80 (H-8), 5.40 (CH_2), 3.74 (NCH_3), 2.59 (CCH_3); ^1H NMR (Me_2SO-d_6, D_2O with excess CF_3COOH) δ8.93, 8.22 (H-2, H-6'), 8.68 (H-8), 5.52 (CH_2), 3.73 (NCH_3), 2.61 (CH_3). Anal. Calcd for $C_{12}H_{15}N_8ClO_4$: C, 33.88; H, 4.09; N, 30.22. Found: C, 33.97; H, 4.11; N, 30.21. The large change in chemical shift of H-8 on protonation is expected;

our values are to be compared with those of δ8.63 (H-2) and 8.58 (H-8)for 3-methyladeninium ion in aqueous acid.[17] Adenine was deuterated at position 8 by heating in D_2O at 100 °C for 8 h.[13]

Bis[(4-amino-1,2-dimethyl-5-pyrimidinio)methyl]amine Diperchlorate VI. A suspension of 0.950 g (1.98 mmol) of 1'-methylthiaminium diperchlorate[5] 1.4 mL (10 mmol) of 2,4,6-trimethylpyridine, and 0.26 g (2.2 mmol) of ammonium perchlorate in 20 mL of methanol was heated at reflux for 18 h. The filter cake was washed with ethyl acetate to give 0.368 g (0.75 mmol, 76%) of product, mp 259-261 °C dec. Recrystallization from water gives the analytical sample of VI: mp 261-264 °C dec; ^1H NMR (Me_2SO-d_6, Me_4Si) δ9.0, 8.3 (NH_2), 8.2 (6-H), 3.8 (NCH_3), 3.6 (CH_2), 3.3 (NH and HOD), 2.6 (CCH_3); ^{13}C NMR δ162.4, 161.4 (C-2 and C-4), 146.1 (C-6), 113.9 (C-5), 44.9 (CH_2), 41.6 (NCH_3), 21.4 (CCH_3). Anal. Calcd for $C_{14}H_{23}N_7Cl_2O_8$: C, 34.44; H, 4.75; N, 20.08. Found: C, 34.30; H, 4.74; N, 19.96.

Nuclear Overhauser Effects. A 50-mg suspension of adenosine derivative III was made to undergo hydrogen-deuterium exchange (OH and some CCH_3) by brief heating in 5 mL of D_2O. Solvent was removed under reduced pressure. The cycle was repeated four times. To a 1% solution of this deuterated III in D_2O was added 0.5 μL of $C_6H_5CH_2OD$ prepared by exchanging the hydroxyl proton in D_2O-DCl, followed by fractional distillation of ether extracts. The solution was filtered through a 1-μm Millipore filter into an NMR tube

and degassed by five freze-thaw-pump cycles. The tube was sealed under vacuum. The NMR tube was soaked in 0.01 M EDTA for 24 h to remove any paramagnetic ions, rinsed with D_2O, and oven dried.

NMR spectra were recorded on a FT JEOL FX-100Q spectrometer. Data were recorded when the CH_2 group was irradiated and also when the sample was irradiated off resonance with a second signal located 500 Hz upfield from the CH_2 signal. The cycle of irradiation on and off resonance was repeated four times; the data were averaged. Signals from the three aromatic protons and the phenyl group of the benzyl alcohol internal standard were processed according to peak heights and areas. The height of the lowest field imidazole-bonded proton essentially was the same during resonance and off resonance. But the height of the signal associated with the proton bonded to the pyrimidine ring of adenosine showed a large NOE enhancement of 33%. The remaining aromatic signal sharpened due to decoupling and possibly increased in intensity due to an NOE, but evaluation is difficult due to the large change in line width associated with decoupling.

The areas of the peaks of interest also were determined by planimetry after the peaks were recorded at a 66.4-Hz sweep width. Areas of the two low-field aromatic protons depend on how a base line is drawn because they do not show base-line separation. The area of the lowest field imidazole signal increased by <3% on irradiation of the CH_2 group, that for the pyrimidine ring of adenine increased by 17-18%, and that for the remaining proton showing decoupling increased

by 9-12%. With the exception of the latter signal, line-width changes for the other two aromatic protons were no more than 2%.

Noteworthy is our preliminary experiment which in a few minutes demonstrated an NOE and gave us a proof of structure. A sample of III in D_2O was routinely prepared. Irradiation of the CH_2 signal with a Varian 360L spectrometer showed that the intensity of the proton bonded to the pyrimidine ring of adenosine increased while that of the imidazole did not; the remaining aromatic proton was decoupled. The more elaborate experiment was then performed as a rigorous check.

In order to check assignments for the aromatic protons in III the H-1′ signal of ribose was irradiated. Because the signal due to CH_2 is close, the irradiating power was systematically varied in order to minimize irradiating this neighbor as well. The signal at lowest field showed NOE enhancement first and others as the irradiating power increased. This lowest field aromatic proton signal must be associated with the imidazole proton. The same conclusion was reached from a deuteration experiment.

Fluorescence Measurements. Fluorescence spectra were recorded on a Perkin-Elmer MPF-44A; they are uncorrected. A 2.4×10^{-3} M solution of III in pH 9.2 borate buffer was prepared by weight. Dilutions with buffer and/or water gave other samples. Spectral data are reported in the text. A 5×10^{-10} M sample which stood under air at room temperature for 1 week showed about a 3-fold reduction in intensity.

Several reaction mixtures were checked to determine whether fluorescent materials are formed. (a) 1'-Methylthiaminium diperchlorate (0.2 M) was heated at 65 °C for 30 h in 80% methanol-20% Me_2SO. No fluorescence was detected. (b) Repetition with prior addition of 2,4,6-trimethylpyridine gave a solution which on 10^3-fold dilution with water had excitation and emission bands at 361 and 413 nm, respectively. (c) A third sample containing the thiamine, the pyridine, and adenine was heated under the same conditions. Following 10^5-fold dilution with water, excitation (391 nm) and emission [410, 428 (shoulder)] were observed. Adenine may form a tetracyclic product related to III in a small amount.

Acknowledgement. This work was kindly supported in part by Grant AM 1744 from the National Institutes of Arthritis, Metabolism, and Digestive Diseases and by a University of Florida Biomedical Research Grant. Dr. G. Uray and Dr. B. Langhammer kindly provided the systematic name for III.

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BIOGRAPHICAL SKETCH

Thomas D. Baugh was born on August 19, 1953, in Big Bear, California. He graduated from San Gorgonio High School in June, 1971. In June, 1975, he received the degree of Bachelor of Science with a major in chemistry from the California State College at San Bernardino.

He enrolled in the Graduate School of the University of Florida in September, 1975. While pursuing his work toward the degree of Doctor of Philosophy, Mr. Baugh was a teaching assistant in both general and organic chemistry, and also NMR and mass spectrometer technician for the Chemistry Department.

Thomas D. Baugh is married to the former Teresa Mofield and is the father of Molly Kate. He is a member of the American Chemical Society.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John A. Zoltewicz, Chairman
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Merle A. Battiste
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

William R. Dolbier, Jr.
Professor of Chemistry

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William Weltner, Jr.
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Biochemistry and
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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1982

Dean for Graduate Studies
and Research